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Design and Synthesis of Diazepin-Steroid Derivative using Some Strategies

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Abstract:

Background: Several steroid derivatives have been prepared using different protocols. The aim of study involved the synthesis of a diazepin-steroid derivative using androsterone as chemical tool.

Methods: Diazepin-steroid derivative was prepared by a series of reactions that involve; 1) Protection of the hydroxyl group from androsterone with tert-Butyldimethylsilyl chloride to form (3*R*,10*S*,13*S*)-3-((*tert*-butyldimethylsilyl)oxy)-10,13-dimethylhexadecahydro-17H-cyclopenta[*a*]phenanthren-17-one (3-TBDS-androst-17-one); 2) catalytic α-hydroxylation of 3-TBDS-androst-17-one for preparation of 3-TBDS-16-hydroxy-steroid-17-one; 3) Reaction of 3-TBDS-16-hydroxy-steroid-17-one with *p*-nitrobenzoyl azide to form 3-TBS-17-oxo-steroid-triazadienium; 4) synthesis of a triazol-steroid derivative by reaction of 3-OTBS-17-oxo-steroid-triazadienium with 1-hexyne; 5) Removal of tert-Butyl-dimethyl-silanyloxy of the triazol-steroid derivative with hydrofluoric acid to form 16-triazole-3-hydroxy-steroid-17-one; 6) Reaction of 16-triazole-3-hydroxy-steroid-17-one with dimethyl sulfoxide to form the 16-triazole-steroid-3-carbaldehyde derivative; 7) synthesis of a enone-steroid derivative by the reaction of 16-triazole-steroid-3-carbaldehyde with acetophenone; 8) Reaction of ethylenediamine with the enone-steroid derivative for synthesis of a diazepin-steroid derivative. The structure of all compounds obtained was confirmed by spectroscopic and spectrometric methods.

Results: The ^{1}H NMR spectrum of diazepin-steroid shows signals at 0.85 and 1.06 ppm for methyl groups bound to steroid nucleus; at 0.90 for methyl group of arm bound to triazole ring; at 0.93-1.04, 1.14-1.36, 1.44-1.74, 1.90, 2.30, 2.60-2.80 and 5.40 ppm for steroid moiety; at, 1.40, 1.80 and 2.44 ppm for methylene groups of arm bound to triazole ring; at 3.10, 3.12, 3.60 and 3.82 ppm for methylene bound to both amino and imino groups; at 3.22-3.56, 3.66 and 4.80 ppm for diazepine ring; 3.10, 3.12, 3.60 and 3.82 ppm for methylene groups bound to both amino and imino groups; at 5.60 ppm for amino groups; at 6.84 ppm for triazole ring; at 6.62-6.80 and 7.34-7.90 ppm for phenyl groups. The ^{13}C NMR spectra displays chemical shifts at 14.30 ppm for methyl group of arm bound to triazole ring; at 11.66 and 16.30 ppm for methyl groups bound to steroid nucleus; at 20.62, 27.60-36.70, 41.20, 42.58, 46.70-48.88, 54.32 and 55.00 and 82.00 ppm for steroid moiety; at 24.18-27.16 ppm for methylene groups of arm bound to triazole ring; at 115.70, 142.60 ppm for triazole ring; at 41.00, 41.32, 54.46, 57.77 and 163.18-165.30 ppm for methylene groups bound to both amino and imino groups; at 44.38, 53.00 and 95.60 ppm for diazepine ring; at 116-138.30 and 154.40 ppm for phenyl groups; at 148.50 and 172.62 ppm for imino groups. In addition, the presence of compound diazepin-steroid was confirmed with mass spectrum which showed a molecular ion at *m/z* 773.54.

Conclusions: In this study is reported a straightforward route for synthesis of a Diazepin-steroid derivative using some strategies. The proposed methods offer some advantages such as simple procedure and ease of workup.

Keywords: Androsterone, diazepin, steroid, synthesis.

1. INTRODUCTION

Since several years ago, have been prepared several steroid derivatives; for example, the synthesis of 3-Ethylenedioxyandrostane-6,17-dione by the reaction of androstan-3,6,17-trione with *p*-toluenesulfonic acid in presence of 2-methyl-2-ethyl-l,3-dioxolane [1]. Other study showed the preparation of 17β-Hydroxy-l7α-methyl-5β-androstan-3-one by the reaction of 17β-hydroxy-l7α-methylandrostan-4-ene-3-one with palladium in basic medium [2]. In addition, other report indicated the reaction of 16-Benzylidene-3-hydroxy-androstan-17-one with fluoroace- tic anhydride to form (8β-9α,14α)-3β-trifluoroacet-16(E)-aryl-methylene-5α-androstan-

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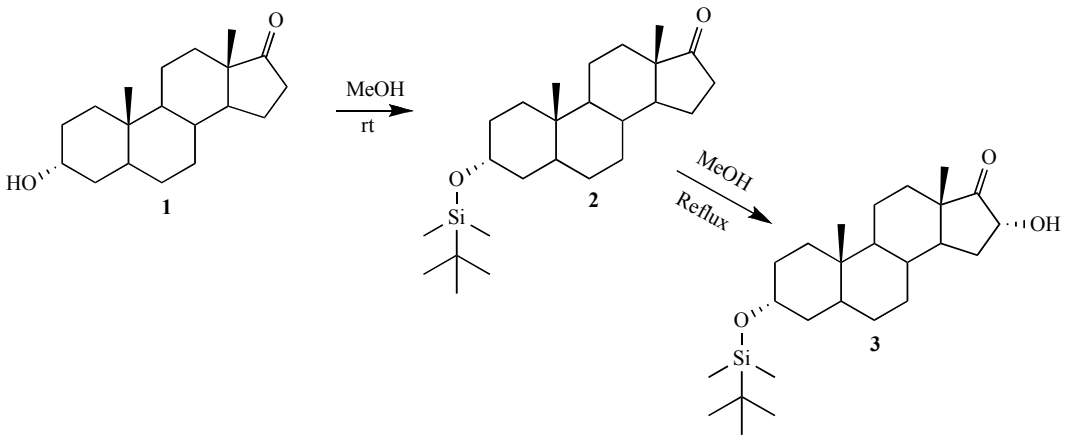

Fig. (1). Synthesis of 3-*tert*-butyldimethylsilyloxy-androst-17-one (**3**). The first stage was achieved by the reaction of androsterone with tert-Butyldimethylsilyl chloride to form the compound 3-TBDS-16-hydroxy-steroid-17-one (**2**). After, **2** was reacted with *N*-Bromosuccinimide/ Dimethyl sulfoxide for preparation of **3**.

17-one [3]. Other data [4] showed the synthesis of 3β-hydroxy-17-hydrazone derivative by the reaction of 3β-hydroxy-17-iodo-13α-androstan-5,16-diene with hydrazine in the presence of barium oxide. Additional, a steroid derivative [5] (17β-cyano-17α-hydroxyandrostan-4-en-3-one was prepared by the reaction of androstan-4-one-3,17-dione with potassium cyanide. Another study [6] showed the Bromination of 2α-methylandrostan-17β-ol-3-one to form the androgen derivative (2-Bromo-17-hydroxy-androstan-3-one). Other study [7] showed the preparation of an androgen-polymer by coupling the compound 17β-Hydroxy-4-androstan-3-one to the dextran. Additionally, a report [8] showed the synthesis of 3β-Hydroxy-5α-androstan-17-cyanoacetyl hydrazone by the reaction of 3β-hydroxy-5α-androstan-17-one with cyanoacetyl hydrazine. In addition, a report indicates the preparation of 1α-Hydroxyhexyl-3-oxo-5α-androstane-17β-yl benzoate by the reaction of 1α-TBSO-hexyl-3-oxo-5α-androstane-17β-yl benzoate with tetrabutyl-ammonium fluoride [9]. Recently [10], 17β-[(t-butyldimethylsilyl)oxy]androst-4-en-3-one reacted with thiourea to form the compound 3-(terbuthyl-dimethyl-silanyloxyl)-5b,3a-Dimethyl-octahydro-indeno[4,5-d]10,12-diaza-tricyclo [7.3.1.01,6]tridecan-9-thione. All these experimental results show several procedures which are available for the synthesis of some steroids derivatives; nevertheless, expensive reagents and special conditions are required. Therefore, The aim of study involved the synthesis of a diazepin-steroid derivative using androsterone as chemical tool.

2. RESULTS AND DISCUSSION

In this study, an androsterone derivative was prepared using some strategies; the first stage was accomplished by protecting of the hydroxyl group from androsterone (Fig. **1**). It is important to mention that several triorganosilyl groups have been employed for the protection of hydroxyl groups such as *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl [11]; in this study, androsterone was made reacting with *tert*-butyldimethylsilyl chloride to form the compound **2**; it is noteworthy that with this method there are very yielding. The ^{1}H NMR spectrum of **2** shows signals at 0.06 and 0.90 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment; at 0.87 and 0.91 ppm for methyl groups bound to steroid nucleus; at 0.89 and 1.00-3.54 ppm for steroid moiety. The ^{13}C NMR spectra display chemical shifts at -4.50, 18.20 and 25.92 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 13.68 and 23.40 ppm for methyl groups bound to steroid nucleus; at 20.20-21.59 and 26.68-72.79 ppm for steroid moiety; at 221.06 ppm for carbonyl group. Finally, the presence of compound **2** was confirmed with mass spectrum which showed a molecular ion at *m/z* 404.31.

The second stage was achieved by a catalytic α-hydroxylation of ketone involved in the compound **2** to form **3** (Figs. **1** and **2**); there are several reports [12] for preparation of α-Hydroxylation of ketones using Osmium tetroxide [12], Iodine [13, 14] and under $CuBr_2$ or HBr/DMSO systems [15]. Analyzing all these data in this study, the compound **3** was prepared by the reaction of **2** with *N*-Bromosuccinimide in DMSO. It is noteworthy that mechanism involved in the α-hydroxylation of ketone involves a substitution reaction in which the nucleophile displaces to bromine (leaving group) by reaction with the carbocation formed. This phenomenon is carried out by the least hindered face which is conditioned by the presence of methyl group bound to steroid nucleus and the cyclopentanone. After, a series of stages are performed to form the α-hydroxilation of ketone. The ^{1}H NMR spectrum of **3** shows signals at 0.07-0.88 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment; at 0.90 and 1.01 ppm for methyl groups bound to steroid nucleus; at 0.93 and 1.04-4.40 ppm for steroid moiety; at 5.35 ppm for hydroxyl group. The ^{13}C NMR spectra displays chemical shifts at -4.50, 18.22 and 26.00 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 16.36 and 23.35 ppm for methyl groups bound to steroid nucleus; at 20.20, 26.68-72.82 ppm for steroid moiety; at 212.56 ppm for carbonyl group. Finally, the presence of compound **3** was confirmed with mass spectrum which showed a molecular ion at *m/z* 420.30.

The third stage involves the synthesis of **4** *via* displacement of nitro group from *p*-nitrobenzoyl azide (Figs. **3** and **4**). It is noteworthy that there are several methods for displacement of nitro groups by methoxy groups [16], fluoride ion [17], nitropropane or nitrocyclohexanone [18], sodium

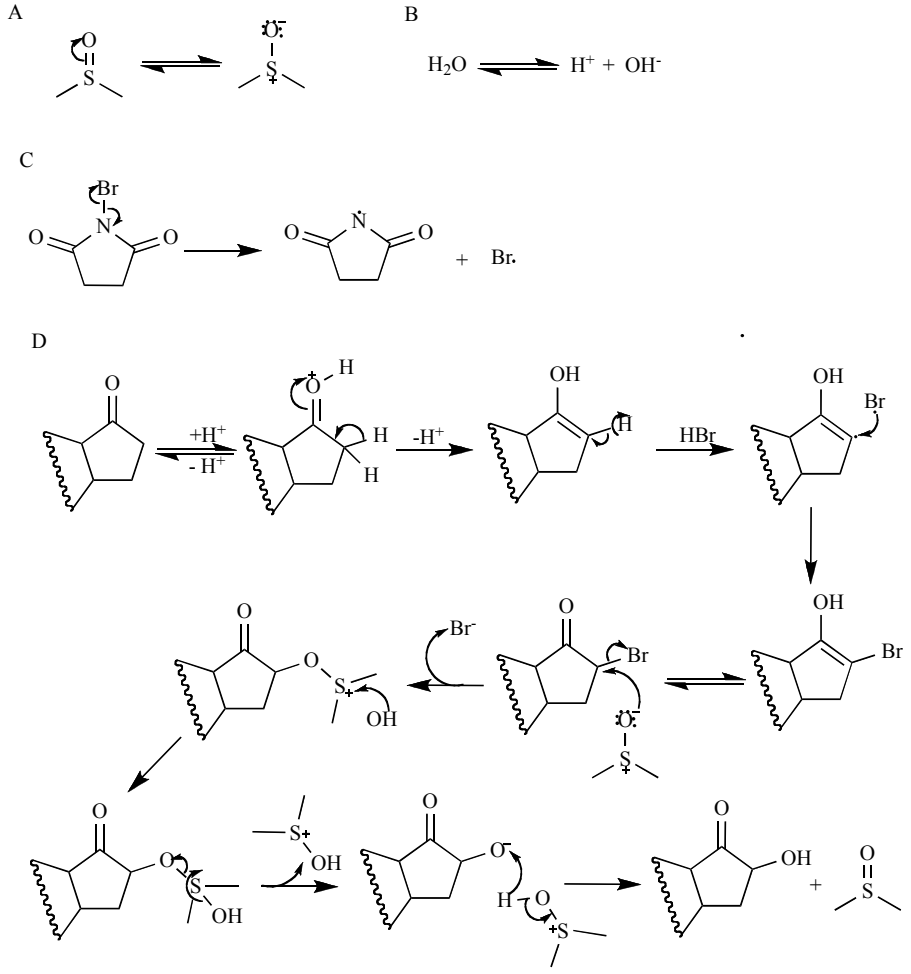

Fig. (2). Mechanism involved in the α-hydroxilation of ketone.

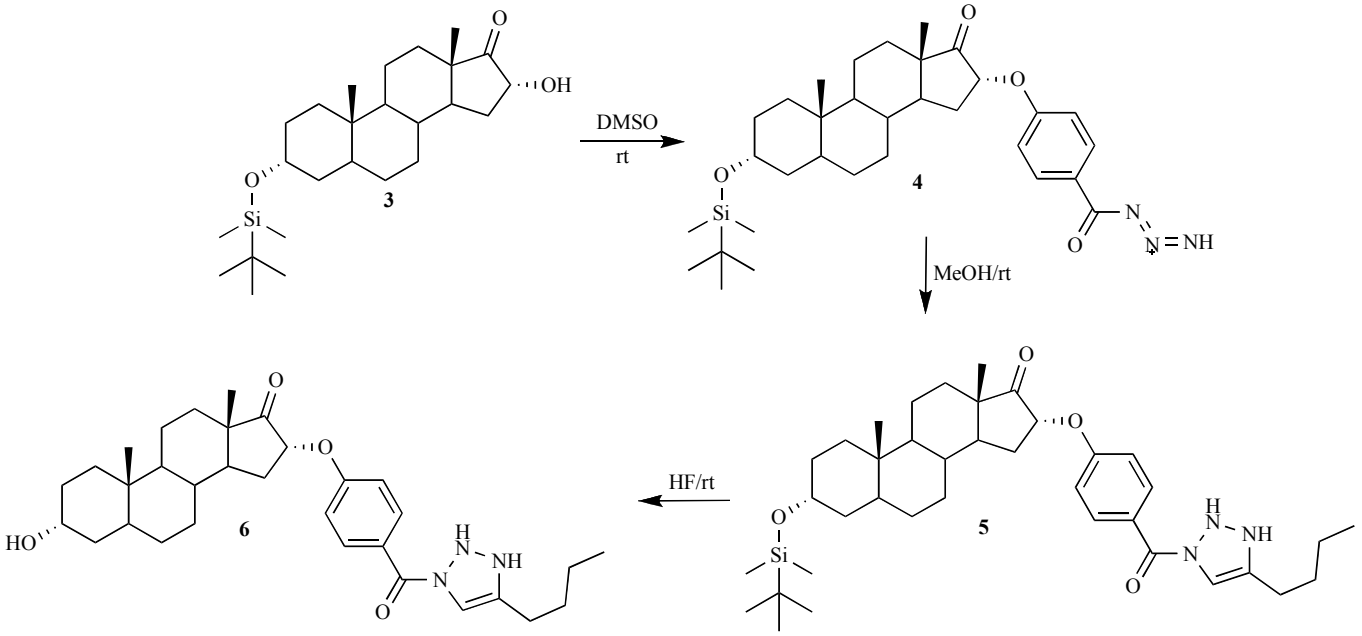

Fig. (3). Synthesis of 16-triazole-3-hydroxy-steroid-17-one (**6**). The first stage was achieved by the reaction of **3** with 4-nitrobenzoyl azide to form 3-TBS-17-oxo-steroid-triazadienium (**4**). After **4** was reacted with 1-hexyne to form the triazol-steroid derivative (5). Finally, hydrofluoric acid was used to removal of the tert-Butyl-dimethylsilanyloxy group of **5** to form **6**.

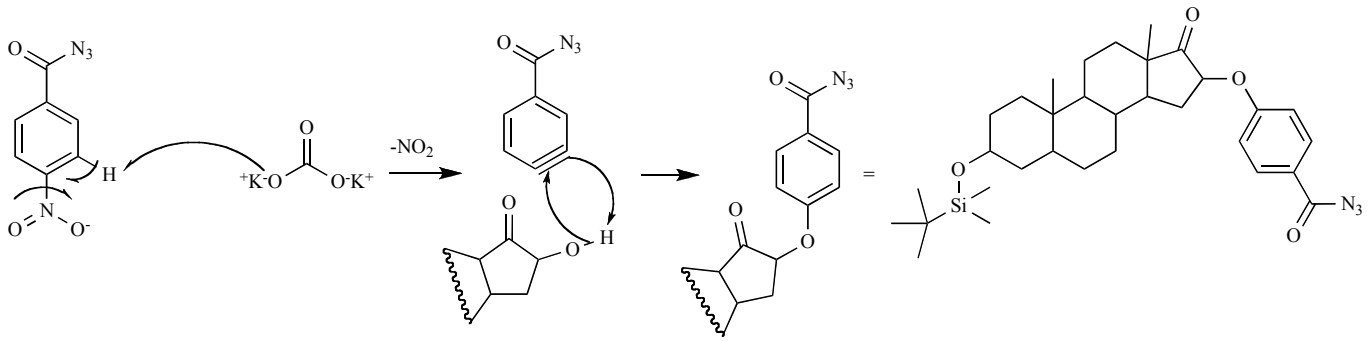

Fig. (4). Mechanism involved in the removal of nitro group of azide and formation of 3-TBS-17-oxo-steroid-triazadienium (**4**).

phenoxide [19], nitrobenzamide in DMSO [20] and others. In this study, the compound **4** was synthetized by the reaction of *p*-nitrobenzoyl azide with the compound **3** in presence of dimetyhyl sulfoxide at mild conditions. The ^{1}H NMR spectrum of **4** shows signals at 0.07-0.88 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment; at 0.92 and 0.96 ppm for methyl groups bound to steroid nucleus; at 0.94 and 1.04-4.60 ppm for steroid moiety; at 7.00-7.70 ppm for phenyl group. The ^{13}C NMR spectra displays chemical shifts at -4.50, 18.31 and 25.98 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 16.40 and 23.42 ppm for methyl groups bound to steroid nucleus; at 20.26 and 26.70-88.26 ppm for steroid moiety; at 113.14-160.92 ppm for phenyl group; at 170.44 ppm for amide group; at 209.68 ppm for ketone group. Finally, the presence of compound **4** was confirmed with mass spectrum which showed a molecular ion at *m/z* 565.33.

The fourth stage (Fig. **3**) was achieved by the synthesis of a triazole derivative (**5**). There are several methods for preparation of triazole rings using thiocarbohydrazide/oxyacetic acid [21], bromomagnesium acetylides/azides [22], alkyne/azides [23, 24] and others. Therefore, in this study **5** was prepared by the reaction of **4** with 1-hexyne. The ^{1}H NMR spectrum of **5** shows signals at 0.07-0.88 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment; at 0.91 and 0.96 ppm for methyl groups bound to steroid nucleus; at 0.93 ppm for methyl group of arm bound to triazole ring; at 0.95, 1.04-1.40, 1.50-1.88, 2.20, 3.56-4.60 ppm for steroid moiety; at, 1.42, 1.90 and 2.50 ppm for methylene group of arm bound to triazole ring; at 5.88 for triazole ring; at 7.00-7.70 ppm for phenyl group; at 9.20 for amino groups. The ^{13}C NMR spectra displays chemical shifts at -4.50, 18.31 and 25.98 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 16.40 and 23.42 ppm for methyl groups bound to steroid nucleus; at 14.30 ppm for methyl group of arm bound to triazole ring; at 24.20, 26.40 and 27.00 form methylene of arm bound to triazole ring; at 20.26 and 26.70, 28.26-88.26 ppm for steroid moiety; at 113.54 and 140.92 ppm for triazole ring; at 114.76-128.56, 158.38 ppm for phenyl group; at 167.00 for amide group; at 209.68 ppm for ketone group. Finally, the presence of compound **5** was confirmed with mass spectrum which showed a molecular ion at *m/z* 649.42.

The fifth stage was performed by the removal of the *tert*-butyldimethylsilyl of **5** (Fig. **3**) to form the compound **6**; it is important to mention that several reagents have been used to removal of protector groups such as Dimethylaluminiun chloride [25], Palla- dium(II) [26], hydrofluoric acid [27]. In this study, aqueous hydrofluoric acid was used to removal the *tert*-butyldimethylsilyl group. The ^{1}H NMR spectrum of **6** shows signals at 0.88 and 0.98 ppm for methyl groups bound to steroid nucleus; at 0.90 ppm for methyl group of arm bound to triazole ring; at 0.93 and 0.95, 1.06-1.36, 1.44-1.86, 2.22, 3.80-4.60 ppm for steroid moiety; at, 1.40, 1.90 and 2.50 ppm for methylene groups of arm bound to triazole ring; at 5.88 for triazole ring; at 7.00 and 7.70 ppm for phenyl group; at 7.10 for both hydroxyl and amino groups. The ^{13}C NMR spectra displays chemical shifts at 14.38 ppm for methyl group of arm bound to triazole ring; at 16.10 and 16.48 ppm for methyl groups bound to steroid nucleus; at 20.32 and 27.96-88.22 ppm for steroid moiety; at 24.18-27.12 ppm for methylene groups of arm bound to triazole ring; at 113.54 and 140.87 ppm for triazole ring; at 114.67-128.57 and 158.38 ppm for phenyl group; at 167.00 ppm for amide group; at 209.58 ppm for ketone group. Finally, the presence of compound **6** was confirmed with mass spectrum which showed a molecular ion at *m/z* 535.34.

The sixth stage was achieved by the synthesis of a carbaldehyde-steroid derivative (**7**); it is noteworthy that there are several reports on the oxidation of primary alcohols to the form the corresponding aldehydes. These compounds can be prepared with some techniques which are accomplished by stoichiometric amounts of metallic oxidants such as chromium(VI) palladium, rhodium or ruthenium and hydrogen peroxide reagents [28]; However, these reagents may induce risks of toxicity by generation of several substances involved on some reaction mixtures. Therefore, in this study a method previously reported [28] for oxidation of hydroxyl groups was used to formation of 7 by the reaction of 6 with DMSO (Figs. **5** and **6**). The ^{1}H NMR spectrum of **7** shows signals at 0.84 and 0.94 ppm for methyl groups bound to steroid nucleus; at 0.91 ppm for methyl group of arm bound to triazole ring; at 0.88, 1.06-1.36, 1.48-1.84, 1.90-2.20, 252-4.60 ppm for steroid moiety; at, 1.40, 1.88 and 2.50 ppm for methylene groups of arm bound to triazole ring; at 5.88 for triazole ring; at 7.00 and 7.70 ppm for phenyl group; at 9.10 ppm for amino groups; at 9.60 ppm for aldol group. The ^{13}C NMR spectra displays chemical shifts at 14.32 ppm for methyl group of arm bound to triazole ring; at 11.66 and 16.40 ppm for methyl groups bound to steroid nucleus; at 20.23-22.00 and 27.94-88.22 ppm for steroid moiety; at 24.18-27.12 ppm for methylene groups of arm bound to triazole ring; at 113.50 and 140.86 ppm for triazole ring; at

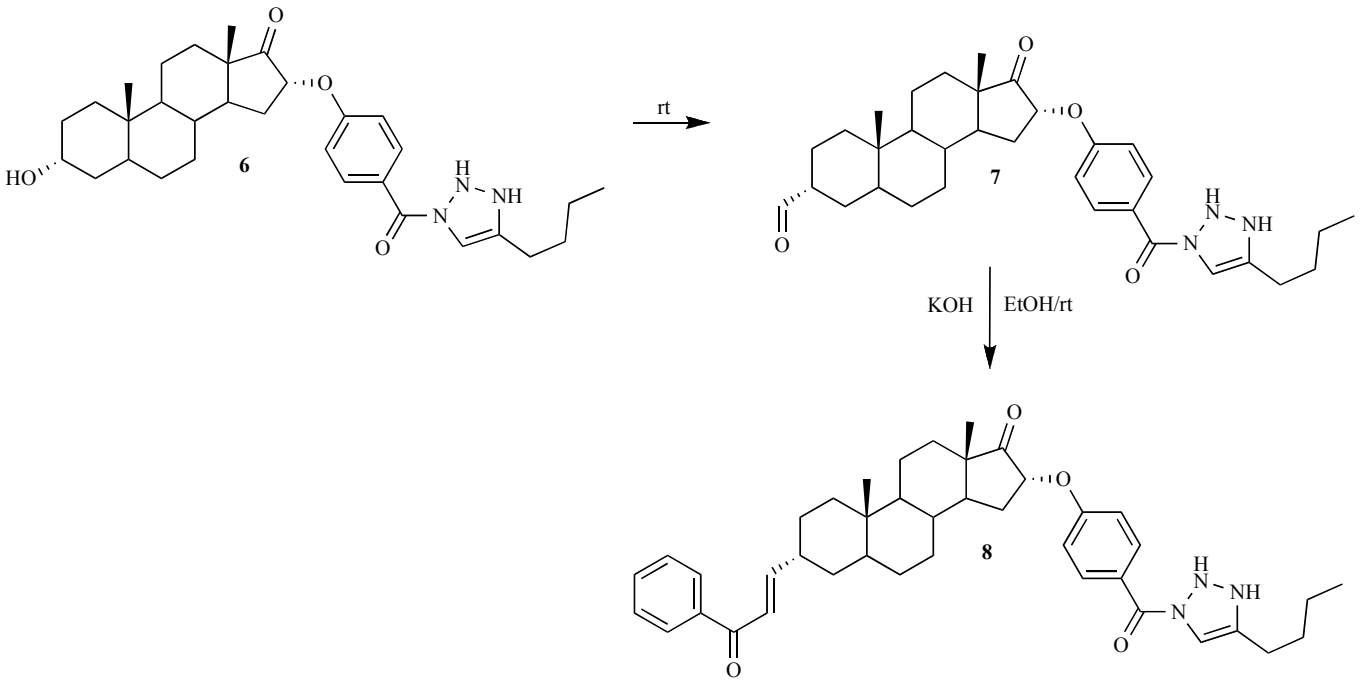

Fig. (5). Synthesis of an enone-steroid derivative (**8**). Reaction of **6** with Dimethyl sulfoxide to form 16-triazole-steroid-3-carbaldehyde derivative (**7**). After **8** was prepared by the reaction of **7** with acetophenone.

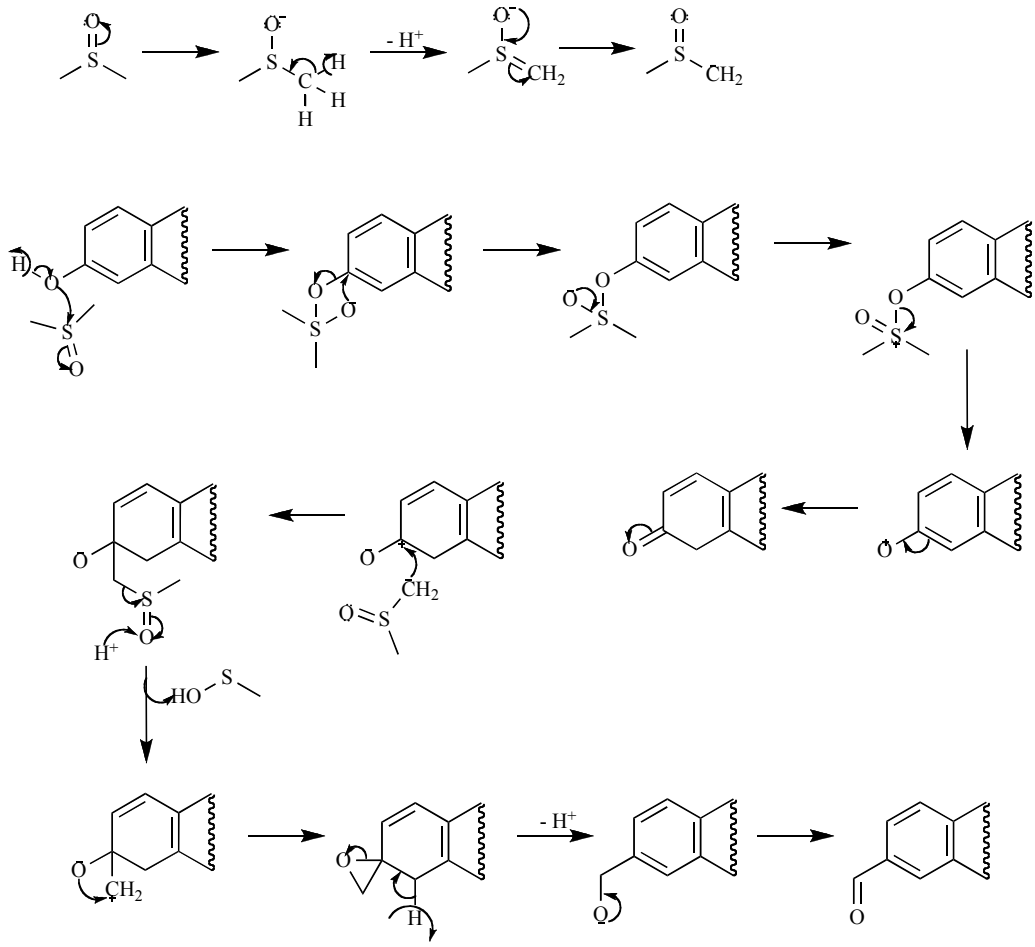

Fig. (6). Mechanism involved in the synthesis of carbaldehyde derivative.

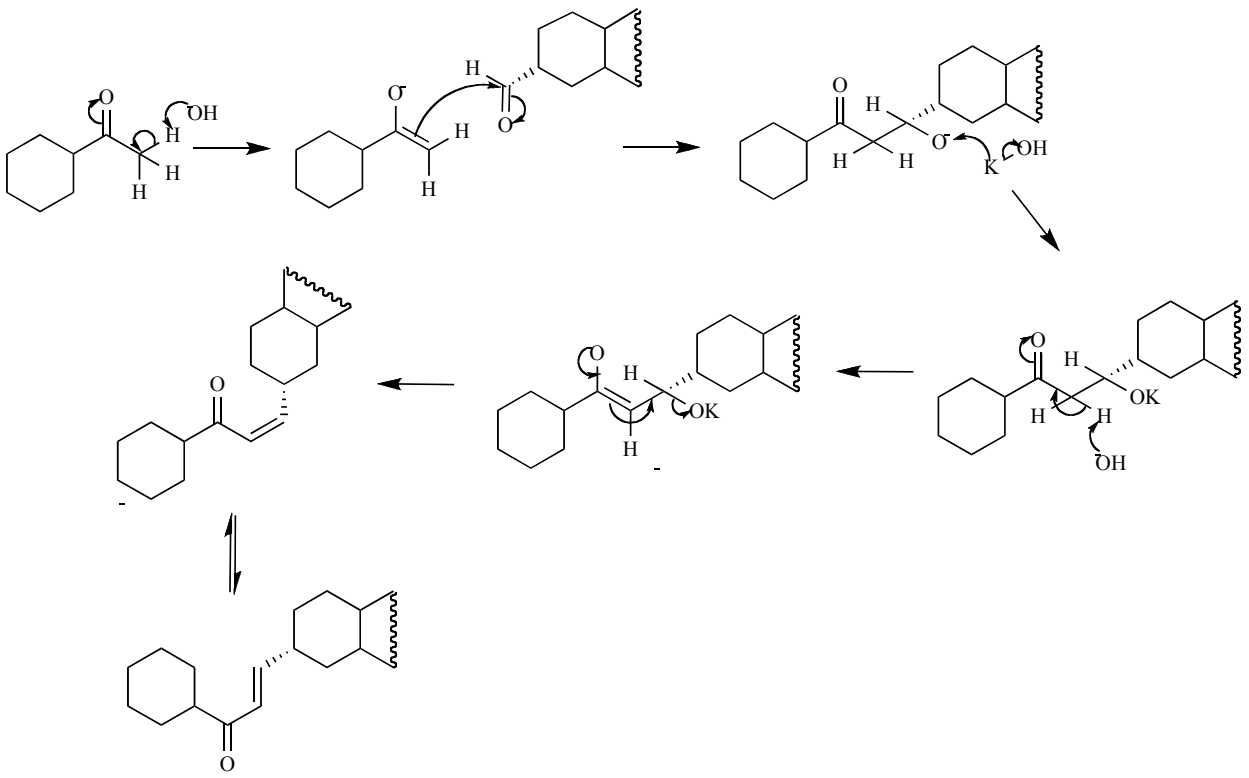

Fig. (7). Mechanism involved in the synthesis of enone-steroid derivative.

114.64-128.50 and 158.39 ppm for phenyl group; at 167.00 ppm for amide group; at 204.88 ppm for aldol group; at 209.56 for ketone group. Finally, the presence of compound **7** was confirmed with mass spectrum which showed a molecular ion at *m/z* 547.34.

The seventh stage was developed by the formation of an enone derivative (**8**). Since years ago, it has increased the interest on the preparations of enone derivatives; using several systems such as aldehyde/ketone [29]; $Pd(DMSO)_2(TFA)_2$/Ketone [30], Ruthenium/alkene [31] and others. Analyzing these data, in this study a new enone-steroid was synthesized by the reaction of **7** with benzaldehyde in presence of potassium hydroxide to form **8** (Figs. **5** and **7**). The ^{1}H NMR spectrum of **8** shows signals at 0.84 and 0.94 ppm for methyl groups bound to steroid nucleus; at 0.91 ppm for methyl group of arm bound to triazole ring; at 0.88, 1.06-1.36, 1.50-1.88, 2.20, 264-4.60 ppm for steroid moiety; at 1.40, 1.90 and 2.50 ppm for methylene groups of arm bound to triazole ring; at 5.88 for triazole ring; at 6.40-6.88 for protons of alkene group; at 7.00 and 7.70 ppm for phenyl group; at 9.10 for amino groups. The ^{13}C NMR spectra displays chemical shifts at 14.30 ppm for methyl group of arm bound to triazole ring; at 11.68 and 16.40 ppm for methyl groups bound to steroid nucleus; at 20.20, 28.10 and 27.94-88.24 ppm for steroid moiety; at 24.18-27.16 ppm for methylene groups of arm bound to triazole ring; at 113.50 and 140.84 ppm for triazole ring; at 114.60, 128.19-137.22 and 158.40 ppm for phenyl group; at 166.98 ppm for amide group; at 124.32 and 150.74 ppm for alkene group; at 188 and 209.56 for ketone groups. Finally, the presence of compound **8** was confirmed with mass spectrum which showed a molecular ion at *m/z* 649.38.

Finally, the height stage was achieved by the formation of a diazepine ring involved in the compound **9**. There are several reports for preparation of diazepine derivatives using some special reagents such as polystyrene supported Sulfonic acid [32], Fe_3O_4/SiO_2 [33], Cooper(I) [34], H_2SO_4/SiO_2 [35], Phosphorus pentasulfide which require special conditions. Analyzing these data in this study **8** was reacting with ethylenediamine to form **9** (Figs. **8** and **9**). This methodology used is a good technique for preparation of diazepine derivatives using an enone and amino groups in acid medium. It is noteworthy that in addition to the ring formation azepine, a new imino group was obtained (**9**). Therefore, the compound **10** is not obtained on the conditions of this study (Fig. **8**).

The ^{1}H NMR spectrum of **9** shows signals at 0.85 and 1.06 ppm for methyl groups bound to steroid nucleus; at 0.90 for methyl group of arm bound to triazole ring; at 0.93-1.04, 1.14-1.36, 1.44-1.74, 1.90, 2.30, 2.60-2.80 and 5.40 ppm for steroid moiety; at, 1.40, 1.80 and 2.44 ppm for methylene groups of arm bound to triazole ring; at 3.10, 3.12, 3.60 and 3.82 ppm for methylene bound to both amino and imino groups; at 3.22-3.56, 3.66 and 4.80 ppm for diazepine ring; 3.10, 3.12, 3.60 and 3.82 ppm for methylene groups bound to both amino and imino groups; at 5.60 ppm for amino groups; at 6.84 ppm for triazole ring; at 6.62-6.80 and 7.34-7.90 ppm for phenyl groups. The ^{13}C NMR spectra displays chemical shifts at 14.30 ppm for methyl group of arm bound to triazole ring; at 11.66 and 16.30 ppm for methyl groups bound to steroid nucleus; at 20.62, 27.60-36.70, 41.20, 42.58, 46.70-48.88, 54.32 and 55.00 and 82.00 ppm for steroid moiety; at 24.18-27.16 ppm for methylene groups of arm bound to triazole ring; at 115.70, 142.60 ppm for triazole

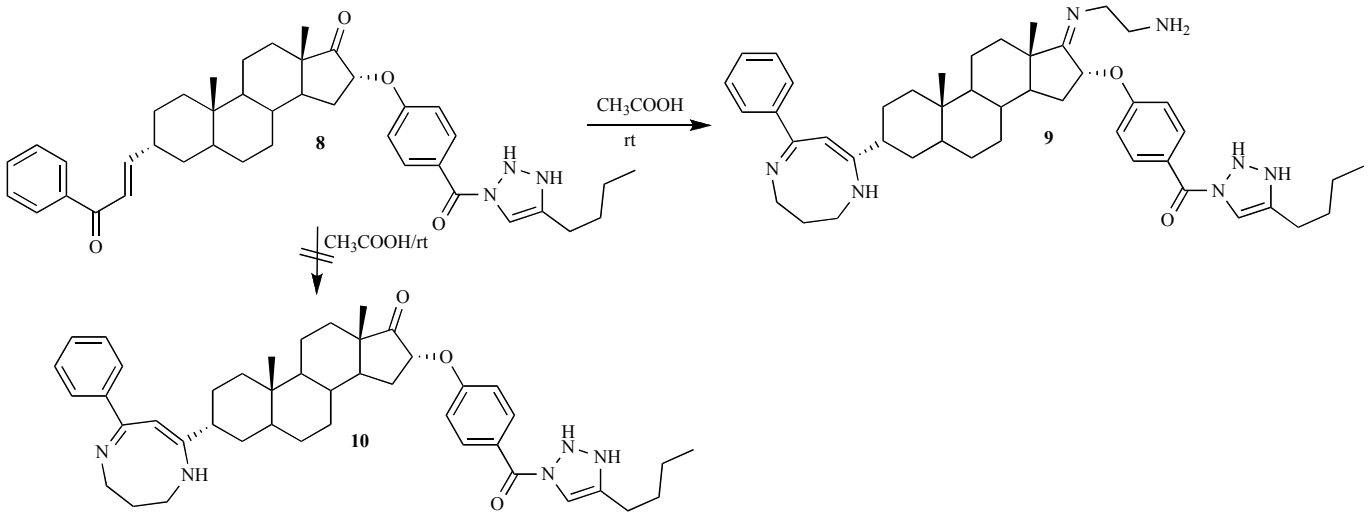

Fig. (8). Synthesis of Diazepin-steroid derivative (**9**). Reaction of the enone-steroid derivative (**8**) with ethylenediamine to form **9**. The compound **10** was not obtained.

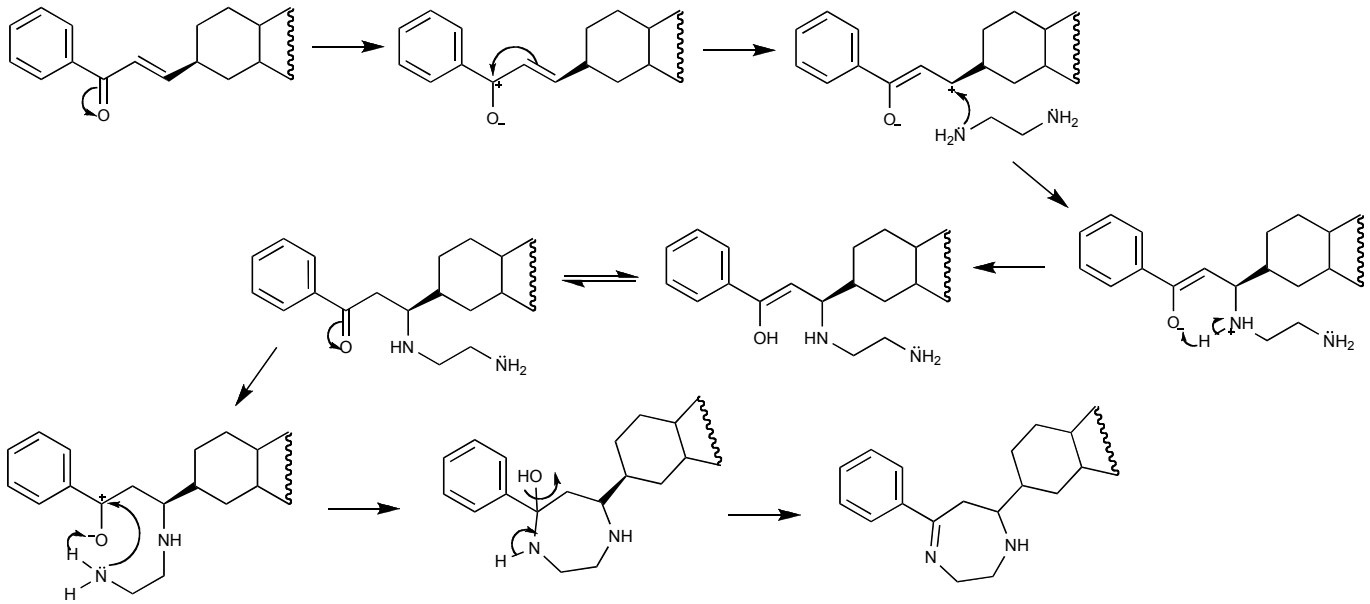

Fig. (9a). Mechanism involved in the formation of diazepin ring of compound **9**.

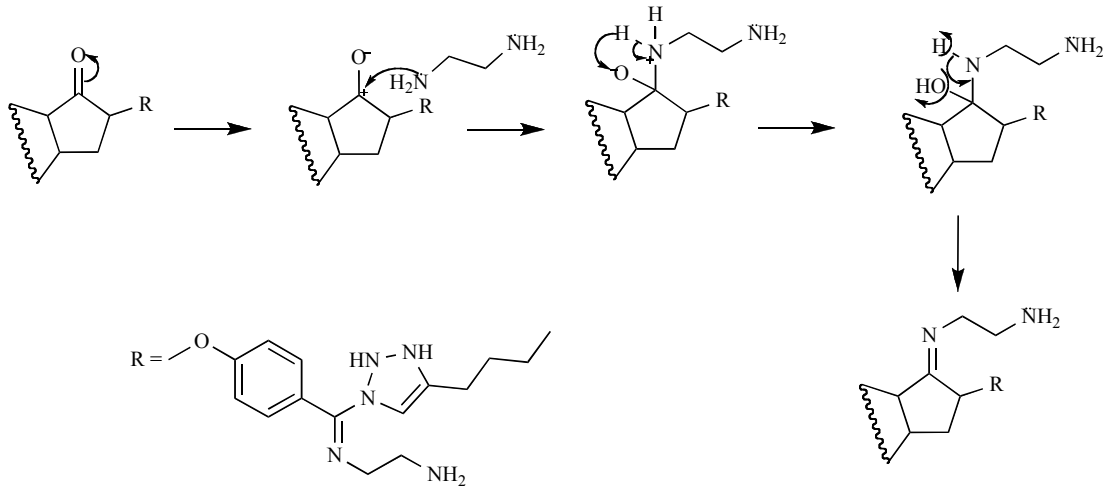

Fig. (9b). Mechanism involved in the formation of imino group of compound **9**.

ring; at 41.00, 41.32, 54.46, 57.77 and 163.18-165.30 ppm for methylene groups bound to both amino and imino groups; at 44.38, 53.00 and 95.60 ppm for diazepine ring; at 116-138.30 and 154.40 ppm for phenyl groups; at 148.50 and 172.62 ppm for imino groups. In addition, the presence of compound **9** was confirmed with mass spectrum which showed a molecular ion at *m/z* 773.54. It is important to mention that compound **10** [(3*R*,10*S*,13*S*,16*R*)-16-(4-(4-butyl-2,3-dihydro-1*H*-1,2,3-triazole-1-carbonyl)phenoxy)-10,13-dimethyl-3-((2*Z*,4*E*)-4-phenyl-1,6,7,8-tetrahydro-1,5-diazocin-2-yl)hexadecahydro-17*H*-cyclopenta[*a*]phenan- theren-17-one) was no formed; this phenomenon possibly is conditioned because the ketone group is more reactive that amide group, which results the formation of imino groups bound to both steroid nucleus and triazole ring.

3. MATERIALS AND METHODS

3.1. General Methods

The reagents used in this study were purchased from Sigma-Aldrich Co. Ltd. The melting point was determined on an Electrothermal (900 model). Infrared spectra (IR) was recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

(3R,10S,13S)-3-((tert-butyldimethylsilyl)oxy)-10,13-dimethylhexadecahydro-17H-cyclopenta[a]phenanthren-17-one (2)

A solution of androsterone (200 mg, 0.69 mmol), *tert*-Butyldimethylsilyl chloride (200 µl, 1.07 mmol) in 3 ml of methanol was stirring for 12 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 86 % of product, m.p. 164-166°C; IR (V_{max}, cm^{-1}): 1720 and 1094; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0.87 (s, 3H), 0.89 (m, 1H), 0.90 (s, 9H), 0.91 (s, 3H), 1.00-1.40 (m, 9H), 1.50-1.66 (m, 5H), 1.70-1.94 (m, 5H), 2.40-3.54 (m, 3H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$ δ_C: -4.50 (**C**-23, **C**-24), 13.68 (**C**-20), 18.20 (**C**-25), 20.20 (**C**-10), 21.59 (**C**-5), 23.40 (**C**-18), 25.92 (**C**-26, **C**-27), 26.68 (**C**-17), 28.02 (**C**-16), 28.20 (**C**-14), 31.50 (**C**-9), 32.33 (**C**-1), 35.17 (**C**-3), 35.63 (**C**-15), 35.70 (**C**-6), 40.18 (**C**-12), 42.36 (**C**-11), 47.64 (**C**-8), 48.30 (**C**-2), 51.49 (**C**-4), 72.79 (**C**-13), 221.06 (**C**-7) ppm. EI-MS *m/z:* 404.31. Anal. Calcd. for $C_{25}H_{44}O_2Si$: C, 74.19; H, 10.96; O, 7.91; Si, 6.94. Found: C, 74.10; H, 10.88.

(3R,10S,13S,16R)-3-((tert-butyldimethylsilyl)oxy)-16-hydroxy-10,13-dimethylhexadecahydro-17H-cyclopenta [a]phenan thren-17-one (3)

A solution of **2** (200 mg, 0.49 mmol), *N*-Bromosuccinimide (100 mg, 0.56 mmol) in 10 ml of Dimethyl sulfoxyde was stirring for 72 h to reflux. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 56 % of product, m.p. 144-146°C; IR (V_{max}, cm^{-1}): 3400, 1718 and 1092; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.07 (s, 6H), 0.88 (s, 9H), 0.90 (s, 3H), 0.93 (m, 1H), 1.01 (s, 3H), 1.04-1.40 (m, 8H), 1.50-1.68 (m, 5H), 1.78-2.00 (m, 6H), 3.58-4.40 (m, 2H), 5.35 (broad, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.50 (**C**-24, **C**-25), 16.36 (**C**-21), 18.22 (**C**-26), 20.20 (**C**-10), 23.35 (**C**-18), 26.00 (**C**-27, **C**-28, **C**-29), 26.68 (**C**-17), 28.16 (**C**-14), 30.52 (**C**-16), 31.26 (**C**-9), 32.33 (**C**-1), 35.17 (**C**3), 35.53 (**C**-5), 35.63 (**C**-15), 40.16 (**C**-12), 42.33 (**C**-11), 48.79 (**C**-8), 49.64 (**C**-2), 50.30 (**C**-4), 67.74 (**C**-6), 72.82 (**C**-13), 212.56 (**C**-7) ppm. EI-MS *m/z:* 420.30. Anal. Calcd. for $C_{25}H_{44}O_3Si$: C, 71.37; H, 10.54; O, 11.41; Si, 6.58. Found: C, 71.30; H, 10.48.

1-(4-(((3R,10S,13S,16R)-3-((tert-butyldimethylsilyl)oxy)-10,13-dimethyl-17-oxohexadecahydro-1H-cyclopenta[a] phenanthren-16-yl)oxy)benzoyl)triaza-1,2-dien-2-ium (4)

A solution of **3** (200 mg, 0.47 mmol), 4-nitrobenzoyl azide (100 mg, 0.52 mmol), in 10 ml of Dimethyl sulfoxide was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 44 % of product, m.p. 254-256°C; IR (V_{max}, cm^{-1}): 3396, 1722, 1094 and 1050; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.07 (s, 6H), 0.88 (s, 9H), 0.92 (s, 3H), 0.94 (m, 1H), 0.96 (s, 3H), 1.04-1.40 (m, 8H), 150-1.88 (m, 10H), 2.20-4.60 (m, 3H), 7.00-7.70 (m, 4H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.50 (**C**-30, **C**-33), 16.40 (**C**-27), 18.31 (**C**-34), 20.26 (**C**-10), 23.42 (**C**-18), 25.98 (**C**-35, **C**-39, **C**-40), 26.70 (**C**-17), 28.26 (**C**-14), 30.56 (**C**-16), 31.70 (**C**-9), 32.33 (**C**-1), 34.60 (**C**-5), 35.20 (**C**-3), 35.63 (**C**-15), 40.21 (**C**-12), 42.28 (**C**-11), 49.24 (**C**-8), 49.60 (**C**-2), 51.88 (**C**-4), 72.85 (**C**-13), 88.26 (**C**-6), 113.14 (**C**-27, **C**-31), 113.22 (**C**-21, **C**-25), 124.80 (**C**-23), 128.18 (**C**-22, **C**-24), 160.92 (**C**-20), 170.44 (**C**-31), 209.68 (**C**-7) ppm. EI-MS *m/z:* 565.33. Anal. Calcd. for $C_{32}H_{47}N_3O_4Si$: C, 67.93; H, 8.37; N, 7.43; O, 11.31; Si, 4.96. Found: C, 67.84; H, 8.30.

(3R,10S,13S,16R)-16-(4-(4-butyl-2,3-dihydro-1H-1,2,3-triazole-1-carbonyl)phenoxy)-3-(tert-butyldimethylsilyl) oxy)-10,13-dimethylhexadecahydro-17H-cyclopenta[a] phenanthren-17-one (5)

A solution of **4** (200 mg, 0.35 mmol), 1-hexyne (90 µl, 0.78 mmol) in 10 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 68 % of product, m.p. 266-268°C; IR (V_{max}, cm^{-1}): 3430, 1720, 1092 and 1050; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.07 (s, 6H), 0.88 (s, 9H), 0.91 (s, 3H), 0.93 (s, 3H), 0.95 (m, 1H), 0.96 (s, 3H), 1.04-1.40 (m, 8H), 1.42 (t, 2H, *J* = 7.40 Hz), 150-1.88 (m, 10H), 1.90 (t, 2H, *J* = 7.40 Hz), 2.20 (m, 1H), 2.50 (t, 2H, *J* = 6.72

Hz), 3.56-4.60 (m, 2H), 5.88 (m, 1H), 7.00-7.70 (m, 4H), 9.20 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.50 (**C**-39, **C**42), 14.30 (**C**-11), 16.40 (**C**-41), 18.31 (**C**-43), 20.26 (**C**-33), 23.42 (**C**-36), 24.20 (**C**-10), 25.98 (**C**-44, **C**-45, **C**-46), 26.40 (**C**-8), 26.70 (**C**-35), 27.00 (**C**-9), 28.26 (**C**-28), 30.56 (**C**-34), 31.70 (**C**-32), 32.33 (**C**-24), 34.60 (**C**-20), 35.20 (**C**-22), 35.63 (**C**-29), 40.18 (**C**-26), 42.28 (**C**-25), 49.24 (**C**-31), 49.70 (**C**-23), 51.88 (**C**-21), 72.85 (**C**-27), 88.26 (**C**-19), 113.54 (**C**-3), 114.76 (**C**-14, **C**-16), 128.40 (**C**-12), 128.58 (**C**-13, **C**-17), 140.92 (**C**-4), 158.38 (**C**-15), 167.00 (**C**-6), 209.68 (**C**-30) ppm. EI-MS *m/z:* 649.42 (M^+10). Anal. Calcd. for $C_{38}H_{59}N_3O_4Si$: C, 70.22; H, 9.15; N, 6.46; O, 9.85; Si, 4.32. Found: C, 70.16; H, 9.08.

(3R,10S,13S,16R)-16-(4-(4-butyl-2,3-dihydro-1H-1,2,3-triazole-1-carbonyl)phenoxy)-3-hydroxy-10,13-dimethyl hexadecahydro-17H-cyclopenta[a]phenanthren-17-one (6)

A solution of **5** (200 mg, 0.30 mmol) in 10 ml of Hydrofluoric acid was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 56 % of product, m.p. 276-278°C; IR (V_{max}, cm^{-1}): 3428, 3398, 1722 and 1052; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.86 (s, 3H), 0.90 (s, 3H), 0.93 (m, 1H), 0.98 (s, 3H), 1.06-1.36 (m, 7H), 1.40 (t, 2H, *J* = 7.40 Hz), 144-1.58 (m, 5H), 1.66-1.86 (m, 6H), 1.90 (t, 2H, *J* = 7.40 Hz), 2.22 (m, 1H), 2.50 (m, 2H, *J* = 6.72 Hz), 3.80-4.60 (m, 2H), 5.88 (m, 1H), 7.00 (m, 2H), 7.10 (broad, 3H), 7.70 (m, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$ δ_C: 14.38 (**C**-11), 16.10 (**C**-33), 16.48 (**C**-39), 20.32 (**C**-33), 24.18 (**C**-10), 26.40 (**C**-8), 27.12 (**C**-9), 27.96 (**C**-35), 30.16 (**C**-28), 30.55 (**C**-34), 33.70 (**C**-32), 34.60 (**C**-20), 34.88 (**C**-29), 35.18 (**C**-22), 35.50 (**C**-24), 36.66 (**C**-26), 42.10 (**C**-25), 49.30 (**C**-31), 49.65 (**C**-23), 51.90 (**C**-21), 69.92 (**C**-27), 88.22 (**C**-19), 113.54 (**C**-3), 114.67 (**C**-14, **C**-16), 128.36 (**C**-12) 128.58 (**C**-13, **C**-17), 140.87 (**C**-4), 158.38 (**C**-15), 167.00 (**C**-6), 209.58 (**C**-30) ppm. EI-MS *m/z:*535.34. Anal. Calcd. for $C_{32}H_{45}N_3O_4$: C, 71.74; H, 8.47; N, 7.84; O, 11.95. Found: C, 71.66; H, 8.40.

(3R,10S,13S,16R)-16-(4-(4-butyl-2,3-dihydro-1H-1,2,3-triazole-1-carbonyl)phenoxy)-10,13-dimethyl-17-oxohexa decahydro-1H-cyclopenta[a]phenanthren-3-carbaldehy- de (7)

A solution of **6** (200 mg, 0.37 mmol) in 10 ml of Dimethyl sulfoxyde was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 69% of product, m.p. 256-258°C; IR (V_{max}, cm^{-1}): 3430, 1738, 1716 and 1052; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.84 (s, 3H), 0.88 (m, 1H), 0.91 (s, 3H), 0.94 (s, 3H), 1.06-1.20 (m, 4H), 1.28-1.36 (m, 4H), 1.40 (t, 2H, *J* = 7.40 Hz), 1.48-1.68 (m, 5H), 1.70-1.84 (m, 4H), 1.88 (t, 2H, J = 7.40 Hz), 1.90-2.20 (m, 2H), 2.50 (t, 2H, *J* = 7.40 Hz), 2.52-4.60 (m, 2H), 5.88 (m, 2H), 7.00-7.70 (m, 4H), 9.10 (broad, 2H), 9.50 (d, 1H, *J* = 7.18 Hz) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$ δ_C: 11.66 (**C**-36), 14.32 (**C**-11**),** 16.40 (**C**-38), 20.23 (**C**-31), 22.00 (**C**-33), 24.18 (**C**-10), 26.36 (**C**-8), 27.12 (**C**-9), 27.94 (**C**-35), 28.00 (**C**-27), 30.54 (**C**-28), 31.70 (**C**-30), 34.58 (**C**-20), 35.20 (**C**-22), 36.28 (**C**-24), 38.97 (**C**-32), 43.10 (**C**-26), 49.32 (**C**-29), 50.71 (**C**-34), 51.13 (**C**-23), 51.88 (**C**-21), 88.22 (**C**-19), 113.50 (**C**-3), 114.64 (**C**-14, **C**-16), 128.50 (**C**-13, **C**-17), 140.86 (**C**-4), 158.39 (**C**-15), 167.00 (**C**-6), 204.88 (**C**-39), 209.56 (**C**-28) ppm. EI-MS *m/z:* 547.34. Anal. Calcd. for $C_{33}H_{45}N_3O_4$: C, 72.36; H, 8.28; N, 7.67; O, 11.68. Found: C, 72.30; H, 8.20.

(3R,10S,13S,16R)-16-(4-(4-butyl-2,3-dihydro-1H-1,2,3-triazole-1-carbonyl)phenoxy)-10,13-dimethyl-3-(E)-3-oxo-3-phenylprop-1-en-1-yl)hexadecahydro-17H-cyclo penta[a] phenanthren-17-one (8)

A solution of **7** (200 mg, 0.36 mmol), acetophenone (68 µl, 0.58 mmol) was added to 10 ml of potassium hydroxide:ethanol (1:9) and stirring for 72 h to room temperature. After the ethanol was evaporated to a smaller volume and the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 43% of product, m.p. 226-228°C; IR (V_{max}, cm^{-1}): 3432, 1720 and 1052; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.84 (s, 3H), 0.88 (m, 1H), 0.91 (s, 3H), 0.94 (s, 3H), 1.06-1.18 (m, 4H), 1.24-1.36 (m, 5H), 1.40 (t, 2H, J = 6.74 Hz), 1.50-1.68 (m, 4H), 1.72-1.88 (m, 5H), 1.90 (t, 2H, J = 6.72 Hz), 2.20 (m, 1H), 2.50 (t, 2H, J = 13.40 Hz), 2.64-4.60 (m, 3H), 5.88 (m, 1H), 6.40-6.88 (m, 2H), 7.00-7.60 (m, 9H), 8.00 (m, 2H), 9.10 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 11.68 (**C**-36), 14.30 (**C**-11), (**C**-38), 16.40, 20.20 (**C**-31), 24.18 (**C**-10), 26.38 (**C**-8), 27.16 (**C**-9), 28.10 (**C**-26), 30.48 (**C**-33), 30.54 (**C**-27), 31.68 (**C**-30), 34.57 (**C**-20), 35.20 (**C**-22), 35.24 (**C**-24), 35.35 (**C**-35), 40.56 (**C**-32), 44.18 (**C**-34), 45.90 (**C**-25), 49.18 (**C**-29), 51.13 (**C**-23), 51.92 (**C**-21), 88.24 (**C**-19), 113.50 (**C**-3, **C**-61), 114.60 (**C**-14, **C**-16), 124.32 (**C**-40), 128.19 (**C**-44, **C**-48) 128.40 (**C**-12), 128.50 (**C**-13, **C**-17), 128.87 (**C**-45, **C**-47), 132.72 (**C**-46), 137.22 (**C**-43), 140.84 (**C**-4), 150.74 (**C**-39), 158.40 (**C**-15), 166.98 (**C**-6), 188.00 (**C**-41), 209.56 ppm. EI-MS *m/z:* 649.38. Anal. Calcd. for $C_{41}H_{51}N_3O_4$: C, 75.78; H, 7.91; N, 6.47; O, 9.85. Found: C, 75.70; H, 7.85.

(4-(((3R,10S,13S,16R,Z)-17-((2-aminoethyl)imino)-10,13-dimethyl-3-((2Z,4E)-4-phenyl-1,6,7,8-tetrahydro-1,5-diazocin-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl)oxy)phenyl)(4-butyl-2,3-dihydro-1H-1,2,3-triazol-1-yl) methanone (9)

A solution of **8** (200 mg, 0.30 mmol), ethylenediamine 5 ml of acetic acid was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 38% of product, m.p. 268-270°C; IR (V_{max}, cm^{-1}): 3430, 3380 and 1050; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.85 (s, 3H), 0.90 (s, 3H), 0.93-1.04 (m, 2H), 1.06 (s, 3H), 1.14-1.36 (m, 5H), 1.40 (t, 2H, J = 7.40 Hz), 1.44-1.74 (m, 9H), 1.80 (t, 2H, *J* = 6.72 Hz), 1.90-2.30 (m, 3H), 244 (t, 2H, *J* = 6.74 Hz), 2.60- 2.80 (m, 2H), 3.10 (t, 2H, *J* = 6.44 Hz), 3.12 (t, 2H, *J* = 6.44 Hz),

3.22-3.56 (m, 3H), 3.60 (t, 2H, *J* = 8.70 Hz), 3.66 (m, 1H), 3.82 (t, 2H, *J* = 6.44 Hz), 4.80 (m, 1H), 5.40 (m, 1H), 5.60 (broad, 7H), 6.62-6.80 (m, 4H), 6.84 (m, 1H), 7.34-7.90 (m, 5H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$ δ_C: 11.66 (**C**-39), 14.30 (**C**-14), 16.30 (**C**-44), 20.62 (**C**-34), 24.18 (**C**-13), 26.18 (**C**-11), 27.16 (**C**-12), 27.60 (**C**-36), 28.20 (**C**-29), 31.43 (**C**-38), 32.50 (**C**-33), 34.00 (**C**-25), 34.20 (**C**-30), 35.22 (**C**-27), 36.70 (**C**-23), 41.00 (**C**-42), 41.20 (**C**-35), 41.32 (**C**-9), 42.58 (**C**-37), 44.38 (**C**-47), 46.70 (**C**-32), 48.88 (**C**-28), 53.00 (**C**-48), 54.32 (**C**-24), 54.46 (**C**-41), 55.00 (**C**-26), 55.77 (**C**-8), 82.00 (**C**-22), 95.60 (**C**-51), 115.70 (**C**-3), 116.00 (**C**-17, **C**-19), 122.74 (**C**-16, **C**-20), 126.40 (**C**-53, **C**-57), 127.60 (**C**-54, **C**-56), 130.10 (**C**-55), 138.30 (**C**-52), 142.60 (**C**-4), 148.50 (**C**-6), 154.40 (**C**-18), 163.18 (**C**-50), 165.30 (**C**-45), 172.62 (**C**-31) ppm. EI-MS *m/z:* 773.54. Anal. Calcd. for $C_{47}H_{67}N_9O$: C, 72.92; H, 8.72; N, 16.28; O, 2.07. Found: C, 72.86; H, 8.66.

4. CONCLUSION

In this study is reported a straightforward route for synthesis of a Diazepin-steroid derivative using some strategies. The proposed methods offer some advantages such as simple procedure and ease of workup.

CONFLICT OF INTEREST

We declare that this manuscript does not have any conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial or otherwise) for its publication.

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Design and Synthesis of New Steroid-Derivatives with Antibacterial Activity on *Salmonella typhi*

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In this study, the following estrogen derivatives were synthesized; oxazin-estradiol-3,17-diol (**6**), oxazine-estradiol-3,17-diyl*bis*-2-chloroacetate (**8**), chloro-acetic acid-estradiol ester (**9**), 3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-estradiol-1,2-diamine (**10**) and 3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-estradiol-chloro-acetamide (**11**) using several strategies. The structure of compounds obtained was confirmed by elemental analysis, spectroscopy and spectrometry data. On the other hand, antibacterial activity of compounds synthesized was evaluated on *Salmonella typhi* with broth dilution methods to determine the minimal inhibitory concentration using gentamycin, ciprofloxacin and cefotaxime as controls. The results indicate that only the compounds **6**, **9**, **10** and **11** decrease the growth of *Salmonella typhi*. The methods used for synthesis of estrogen derivatives offers some advantages such as simple procedure, low cost and ease of workup. In addition, the antibacterial activity showed the compounds **6**, **9**, **10** and **11** depend on chemical structure in comparison with the controls involved. These estrogenic derivatives could be used as a therapeutic alternative for treatment of infectious diseases induced by *Salmonella typhi*.

Keywords: Estrogen derivative, Estradiol, Chloroacetyl chloride, *Salmonella typhi*.

INTRODUCTION

There are reports which indicate that *Salmonella typhi* is a human pathogen that induces several deaths each year [1-3]. Diverse drugs have been used for their treatment. However, some strains of *Salmonella typhi* have induced resistance to chloramphenicol, ampicillin and trimethoprim, streptomycin, sulfonamides and tetracyclines in under developing countries [4,5]. In search of new alternative therapeutics for treatment of resistance exerted by *Salmonella typhi*, have developed several antibacterial drugs. For example, the synthesis of several hydrazones were prepared by reacting isatin and aromatic primary amines/hydrazines [6]. Other data showed that several polysaccharides-tetanus toxoid conjugates induce antibacterial activity on *Salmonella typhi* [7].

On the other hand, also some steroids as potential therapeutic agents have been developed for *Salmonella typhi*; for example, there is a study which showed the synthesis of steroidal thiocarbazone derivatives with antibacterial activity on *Salmonella typhimurium* [8]. In addition, a steroid derivative (cholest-5-en-3-oxazolo) was synthesized and their antibacterial effect was evaluated on *Salmonella typhimurium* [9]. Other data indicate the preparation of a steroid-thiourea derivative with antibacterial effect on *Salmonella typhimurium* [10]. Additionally, other steroid derivatives (3α-hydroxy-23,24-*bis*-norcholane polyamine carbamates) with antibacterial activity on *Salmonella typhimurium* were synthesized [11]. All these experimental results show several procedures which are available for synthesis of several antibacterial steroid-derivatives. Nevertheless, expensive reagents and special conditions are required. Therefore, in this study some steroid derivatives were synthesized using several strategies. It is noteworthy that antibacterial activity of these steroid derivatives on *Salmonella typhi* was evaluated *in vitro* in a bacteria model.

EXPERIMENTAL

The compound 4-[(2-amino-ethylamino)methyl]-13-methyl-7,8,9,11,12,13,14,15,17-decahydro-6*H*-cyclopenta-[a]phenanthrene-3,17-diol (**1**) was synthesized using reported method [12]. The other compounds evaluated in this study were purchased from Sigma-Aldrich Co. Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Infrared spectra were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

Synthesis of 3-(1*H*-naphtho[1,2-e][1,3]oxazin-2(3*H*)-yl)propan-1-amine (3): A solution of 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol), ethylenediamine (50 µL, 0.75 mmol) and formaldehyde (3 mL) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to reduce the volume. After the mixture was diluted with water and extracted with chloroform (Fig. 1). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 54 % of product, m.p.: 100-102 °C; IR (ν_{max}, cm^{-1}): 3380 and 1196; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.68 (t, 2H, *J* = 7.00 Hz), 1.76 (broad, 2H), 2.50 (m, 2H), 2.56 (m, 2H), 4.12-4.86 (m, 4H), 7.04-7.80 (m, 6H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 30.90, 39.22, 50.20, 53.66, 82.00, 111.22, 117.90, 120.36, 123.30, 126.57, 127.90, 128.34, 133.08, 152.32 ppm. EI-MS *m/z:* 214.14 (M^+11). Anal. calcd. for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56, O, 6.60. Found: C, 74.30; H, 7.42.

Synthesis of 13-methyl-4{[2-(1*H*-naphto[1,2-e][1,3]oxazin-2-yl)-ethylamino]methyl}-7,8,9,11,12,13,14,15,16,17-decahydeo-6*H*-cyclopenta[a]phenanthrene-3,17-diol (6)

Method A: A solution of **1** (100 mg, 0.29 mmol), **4** (60 mg, 0.34 mmol) and formaldehyde (3 mL) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 66 % of product (Fig. 2), m.p.: 196-198 °C; IR (ν_{max}, cm^{-1}): 3412, 3380 and 1200; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.62 (s, 3H), 0.80-1.20 (m, 4H), 1.30-1.40 (m, 3H), 1.68- 1.84 (m, 4H), 2.06-2.50 (m, 4H), 2.54 (t, 2H, *J* = 6.90), 2.64 (t, 2H, *J* = 6.90), 3.60 (m 1H), 3.76 (t, 2H, *J* = 12.00), 4.30-5.00 (m, 3H), 5.08 (s, 3H), 5.10 (m, 1H), 6.52-6.80 (m, 2H), 7.00-7.72 (m, 6H) ppm.^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 15.68, 24.20, 25.30, 27.58, 27.68, 32.56, 33.70, 37.20, 44.28, 44.46, 44.52, 47.20, 50.22, 50.76, 55.00, 82.36, 82.40, 111.20, 112.48, 118.4, 120.78, 122.40, 123.3, 126.30, 127.86, 128.20, 128.40, 128.72, 131.7, 131.7, 137.25, 148.98, 151.64 ppm. EI-MS *m/z:* 512.30 (M^+12). Anal. calcd. for $C_{33}H_{40}N_2O_3$: C, 77.31; H, 7.86; N, 5.46, O, 9.36. Found: C, 77.27; H, 7.82.

Method B: A solution of estradiol (100 mg, 0.37 mmol), **3** (90 mg, 0.37 mmol) in formaldehyde (5 mL) was stirring for 72 h to reflux. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (3:1) yielding 44 % of product (Fig. 2). Similar ^{1}H NMR and ^{13}C NMR data were obtained compared with method A.

Synthesis of (13R)-4-((2-(1*H*-naphtho[1,2-e][1,3]-oxazin-2(3*H*)-yl)ethyl)amino)methyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta-[a]phenanthrene-3,17-diyl*bis*(2-chloroacetate) (8): A solution of **6** (250 mg, 0.48 mmol), triethylamine (100 µL, 1.50 mmol) and chloroacetyl chloride (128 µL, 1.60 mmol) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 3). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from hexane:methanol:water (1:3:1) yielding 55 % of product, m.p.: 178-180 °C; IR (ν_{max}, cm^{-1}): 1730, 1210 and 1192; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.82 (s, 3H), 1.12-1.28 (m, 3H), 1.40-1.50 (m, 3H), 1.68-1.74 (m, 4H), 2.12-2.22 (m, 2H), 2.40-2.52 (m, 3H), 2.58 (t, 2H, *J* = 6.90 Hz), 2.64 (t, 2H, *J* = 6.90 Hz), 3.60 (broad, 1H), 3.80 (m, 2H), 4.06-4.10 (m, 4H), 4.30-4.40 (m, 2H), 4.80 (m, 1H), 5.00-5.10 (m, 2H), 6.72-6.80 (m, 2H),, 7.00-7.80 ppm.^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 14.30, 24.58, 25.20, 27.60, 27.70, 30.00, 33.70, 37.20, 40.56, 40.80, 44.00, 44.50, 44.88, 47.30, 50.36, 50.94, 55.10, 82.34, 84.54, 111.20, 118.22, 118.40, 118.66, 120.72, 123.30, 126.30, 127.86, 128.38, 128.80, 130.64, 131.70, 135.52,

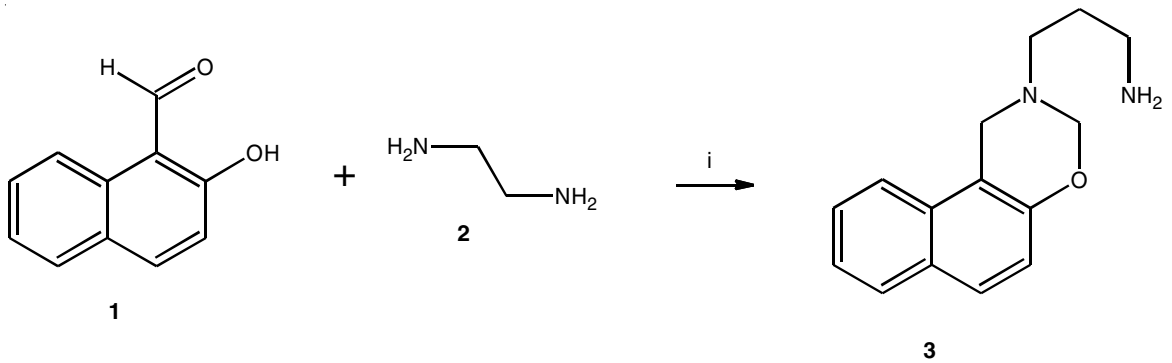


Fig. 1. Synthesis of 3-(1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)propan-1-amine (**3**). Reaction of 2-hydroxy-1-naphthaldehyde (**1**) with ethylenediamine (**2**) to form **3**.i = formaldehyde/room temperature

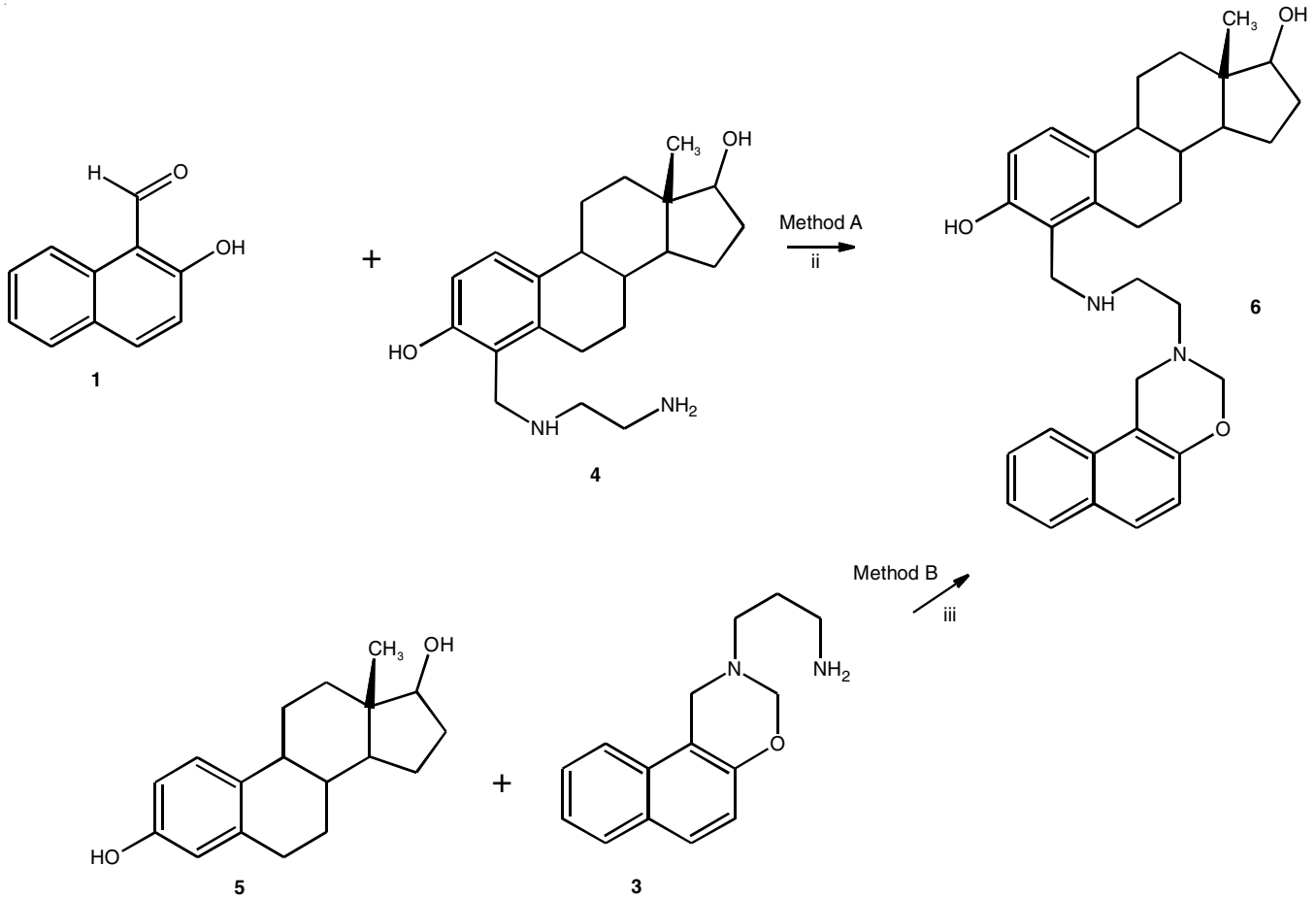
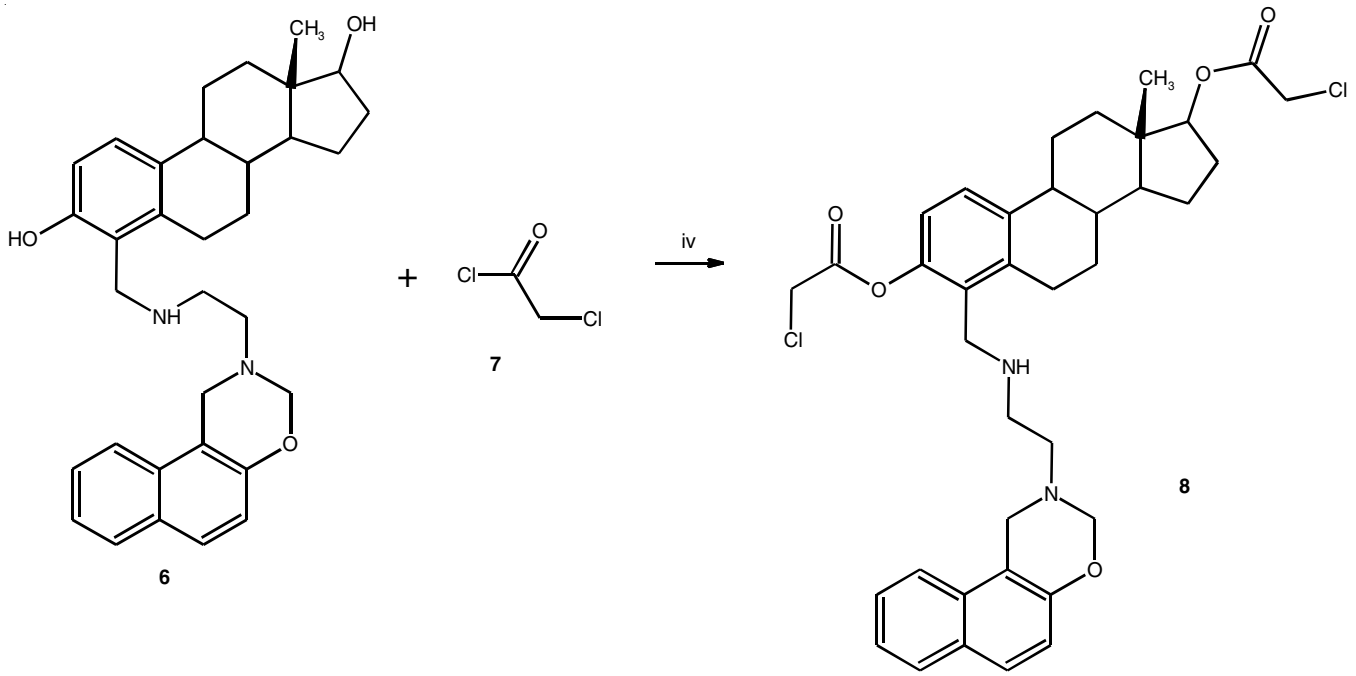


Fig. 2. Synthesis of an estrogen derivative (**6**). The first stage was achieved by reaction of 4-[(2-amino-ethylamino)-methyl]-13-methyl-7,8,9,11,12,13,14,15,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (**4**) with 2-hydroxy-1-naphthaldehyde (**1**) to form **6**. Also **6** was synthesized by the reaction ofestradiol (**5**) with 3-(1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)propan-1-amine (**3**). i = formaldehyde/MeOH/room temperature; ii = formaldehyde/MeOH/reflux



Fi. 3. Chloro-acetic acid 3-(2-chloro-acetoxy)-4-({(2-chloro-3-oxo-cyclobutyl)-[2-(1*H*-naphto[1,2-e][1,3]oxazin-2-yl)-ethyl]amino}methyl)-13-methyl-7,8,9,11,12,13, 14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl ester (**8**). Reaction of the compound **6** with chloroacetyl chloride to form **8**. iii = triethylamine/MeOH/room temperature

138.44,139.50, 144.52, 151.66, 166.74, 168.00 ppm. EI-MS *m/z:* 664.24 (M^+10). Anal. calcd. for $C_{37}H_{42}N_2O_5Cl_2$: C, 66.76; H, 6.36; Cl, 10.65; N, 4.21, O, 12.02. Found: C, 66.70; H, 6.30.

Synthesis of chloro-acetic acid 3-(2-chloro-acetoxy)-4-{[2-(2-chloro-acetylamino)ethylamino]methyl}-13-methyl-7,8,9,1,12,13,14,15,16,17-decahydro-6*H*-cyclopenta-[a]phenanthren-17-yl ester (9): A solution of **4** (200 mg, 0.58 mmol), triethylamine (100 µL, 1.50 mmol) and chloroacetyl chloride (128 µL, 1.60 mmol) in 10 mL of methanol was stirring for 72 h at room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 4). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 56 % of product, m.p.: 268-270 °C; IR (ν_{max}, cm^{-1}): 3310, 1210 and 1192; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.82 (s, 3H), 1.12-1.30 (m, 3H), 1.40-1.50 (m, 3H), 1.68-1.76 (m, 4H), 2.12-2.52 (m, 5H), 2.66 (t, 2H, *J* = 6.44 Hz), 3.38 (t, 2H, *J* = 6.44 Hz), 3.80 (s, 2H), 4.00-4.12 (m, 6 H), 4.82 (s, 1H), 5.76 (broad, 2H), 6.72-6.84 (m, 2H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 14.22, 25.16.00, 27.50, 27.63, 30.00, 33.56, 37.20, 38.46, 40.50, 40.72, 42.36, 44.00, 44.50, 44.76, 50.82, 52.80, 84.56, 118.64, 130.68, 135.58, 138.48, 139.36, 144.36, 162.50, 166.76, 168.08 ppm. EI-MS *m/z:* 572.16 (M^+11). Anal. calcd. for $C_{27}H_{35}N_2O_5Cl_3$: C, 56.50; H, 6.15; Cl, 18.53; N, 4.88, O, 13.94. Found: C, 56.42; H, 6.10.

Synthesis of N-1-[3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-4-ylmethyl]ethane-1,2-diamine (10): A solution of **4** (200 mg, 0.58 mmol) and *tert*-butyldimethylsilyl chloride (200 µL, 1.07 mmol) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 5). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (4:1) yielding 78 % of product, m.p.: 144-146 °C; IR (ν_{max}, cm^{-1}): 3380, 3310 and 1094. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.08 (s, 6H), 0.25 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 0.96 (s, 9H), 1.01-1.90 (m, 10H), 2.08 (m, 1H), 2.20 (broad, 3H), 2.42-2.50 (m, 3H), 2.62 (t, 2H, *J* =5.97 Hz), 2.76 (t, 2H, *J* = 5.97 Hz), 3.50 (m, 1H), 3.62 (m, 2H), 658-6.80 (m, 2H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: -4.50, -4.20, 15.18, 17.76, 18.44, 25.30, 25.54, 25.70,25.74, 27.65, 27.70, 32.97, 35.00, 37.28, 41.52, 43.70, 44.48, 45.88, 51.42, 53.30, 82.56, 115.12, 123.78, 127.20, 131.29, 136.19, 150.76 ppm. EI-MS *m/z:* 572.40 (M^+11). Anal. calcd. for $C_{33}H_{60}N_2O_2Si_2$: C, 69.17; H, 10.55; N, 4.89, O, 5.58; Si, 9.80. Found: C, 69.12; H, 10.48.

Synthesis of N-(2-{[3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-4-ylmethyl]amino}-ethyl)-2-chloro-acetamide (11): A solution of **10** (400 mg, 0.70 mmol), triethylamine (100 µL, 1.50 mmol) and chloroacetyl chloride (128 µL, 1.60 mmol) in 10 mL of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to a smaller volume. After the mixture was diluted with water and extracted with chloroform (Fig. 5). The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (2:1) yielding 52 % of product, m.p.: 238-240 °C; IR (ν_{max}, cm^{-1}): 3310, 1680 and 1096; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.06 (s, 6H), 0..25 (s, 6H), 0.80 (s, 3H), 0.88 (s, 9H), 1.00 (s, 9H), 1.20-1.90 (m, 10H), 2.10-2.50 (m, 4H), 2.72 (t, 2H, *J* = 6.44), 3.40 (t, 2H, *J* = 6.44), 3.50 (m, 1H), 364 (t, 2H, *J* = 12.00), 4.00 (m, 2H), 5.00 (broad, 2H), 6.50-6.80 (m, 2H) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: -4.50, -4.20, 15.18, 17.76, 18.42, 25.30, 25.54, 25.70, 25.68, 25.74, 27.60, 27.70, 32.97, 35.01, 37.28, 38.57, 42.40, 43.70, 44.45, 45.83, 51.49, 52.80, 82.60, 115.09, 124.13, 127.18, 131.30, 136.14, 150.78, 162.56ppm.EI-MS *m/z:* 648.36 (M^+10). Anal. calcd. for $C_{35}H_{61}N_2O_3Si_2Cl$: C, 64.72; H, 9.47; Cl, 5.46; N, 4.31; O, 7.39; Si, 8.65. Found: C, 64.68; H, 9.42.

Antimicrobial activity: The evaluation of antimicrobial effect of the different compounds on *Salmonella typhi* was made by described method [13]. In this method, *Salmonella typhi* was incubated on McConkey agar for 24 h at 37 °C. After some time, it was determined whether growth had taken place or not. In addition, a series of tubes were prepared, the first contained 2 mL of culture medium (tripticasesoye) at double concentration and the remainder (11 tubes), contained

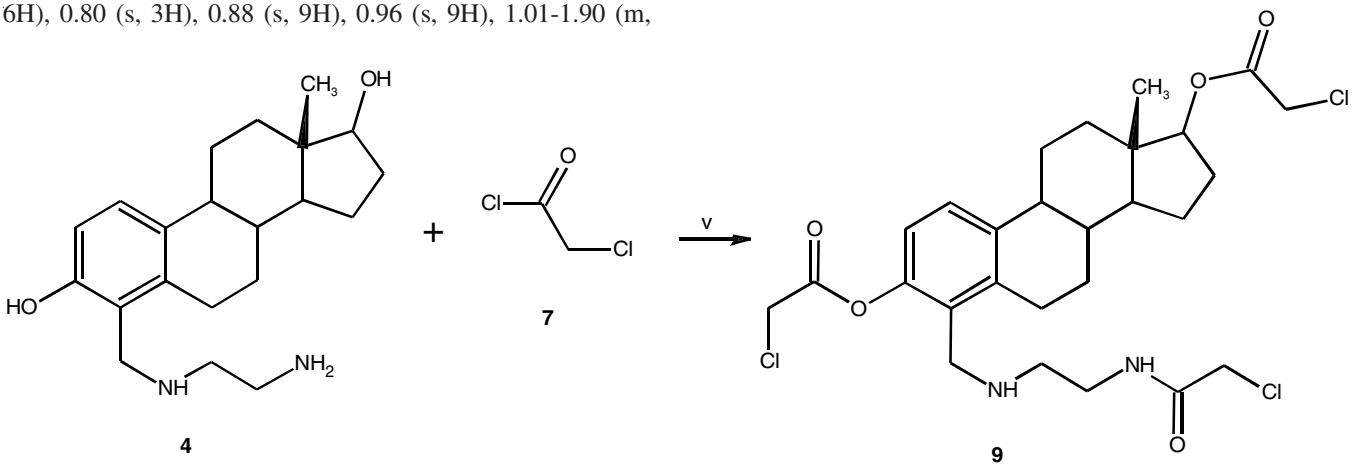


Fig. 4. Synthesis of chloro-acetic acid 3-(2-chloro-acetoxy)-4-{[2-(2-chloro-acetylamino)-ethylamino]-methyl}-13-methyl-7,8,9,1,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-yl ester (**9**). Reaction of compound **4** with chloroacetyl chloride (**7**) to form **9**. iv = triethylamine/MeOH/room temperature

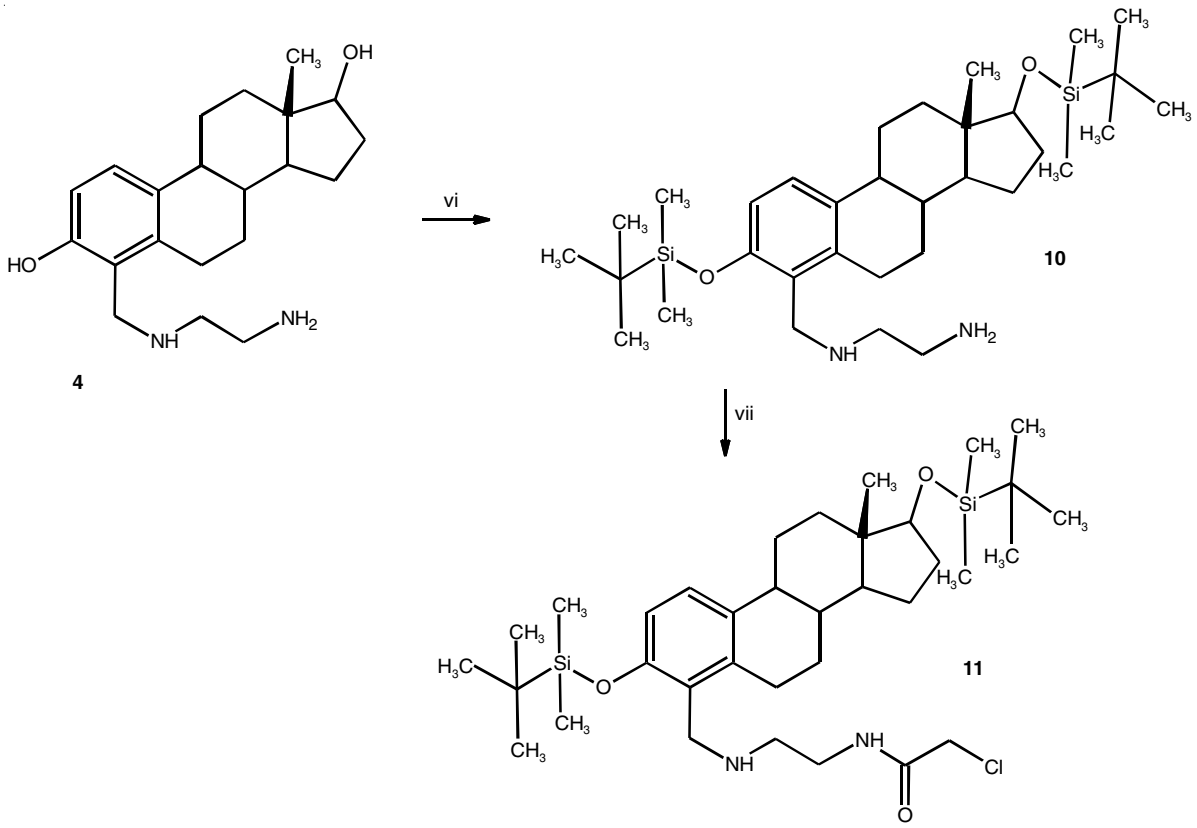


Fig. 5. Synthesis of an estrogen derivative (**11**). The first stage was achieved by reaction of **4** with *tert*-butyldimethylsilyl chloride (vi) to form the compound N-1-[3,17-*bis*-(*tert*-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]-phenanthren-4-ylmethyl]ethane-1,2-diamine (**10**). After, **10** was made reacting with chloroacetyl chloride (vii) to form **11**. vii = triethylamine/MeOH/room temperature

the same quantity of medium at single concentrations. From the first tube (double concentration) an aliquot of 2 mL of the studied compound (1 mg/mL) was added and stirred, from this tube an aliquot of 2 mL was taken and added to the following tube (simple concentration) and the process was successively repeated until the last 2 mL of dissolution had been used up. After this process, each tube was inoculated with 0.1 mL of the bacterial suspension, whose concentration corresponded to Mc-Farland scale (9×10^8 cells/mL) and all the tubes were incubated at 37 °C for 24 h. Subsequently, a loop was taken from each of them and inoculated into the appropriate cultures for different bacterial organisms and were incubated for 24 h at 37 °C. After such time, the minimum inhibitory concentration (MIC) was evaluated to consider the antimicrobial effect of the different compounds. In order to discard the effect of methanol (solvent) on the bacterial species studied, a series of the same number of tubes was prepared in parallel, to which 2 mL of methanol at 60 % was added to the first and corresponding successive dilutions were added in the same way as before. In addition a control series was also performed using distilled water to pH 7.0.

Statistical analysis: The obtained values are expressed as average ± SE [14]. The differences were considered significant when p was equal or smaller than 0.05.

RESULTS AND DISCUSSION

In this study, some antibacterial estrogen derivative was developed using several strategies. The first step was achieved by the synthesis of oxazine-steroid derivative (**3**). It is important to mention that many procedures for the synthesis of oxazine derivatives are available in the literature [15-18]. Nevertheless, expensive reagents and special conditions are required; therefore, in this study the compound **3** was synthesized using Mannich reaction [19]. ^{1}H NMR spectrum of **3** shows signals at 1.68 and 2.50-2.56 ppm for methylene groups bound to both amino groups; at 1.76 ppm for amino group; at 4.12-4.86 ppm for protons of oxazine ring; at 7.07-7.80 ppm for both phenyl groups. ^{13}C NMR spectrum of **3** contains peaks at 30.90-39.22 and 53.66 ppm for methylene groups bound to both amino groups; at 50.20 and 82.00 ppm for carbons of oxazine ring; at 11.22-152.32 ppm for both phenyl groups. Finally, the presence of compound **3** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 214.14.

The second stage was achieved using two methods; in the method A, the compound **6** was synthesized by condensation of an estrogen-derivative with2-hydroxy-1-naphthaldehyde using Mannich reaction [19]. ^{1}H NMR spectrum of **6** shows signals at 0.62 ppm for methyl group bound to steroid nucleus; at 0.80-2.50, 3.60 and 6.52-6.80 ppm for steroid moiety; at 2.54-2.64 ppm for methylene groups bound to both amine groups; at 3.76 for methylene group bound to both amino group and ring A of steroid nucleus; at 4.30-5.00 and 5.10 ppm for oxazine ring; at 5.08 ppm for both hydroxyl and amino groups. ^{13}C NMR spectrum of **3** contains peaks at 15.68 ppm for methyl group bound to steroid nucleus; at 24.20-44.46, 50.76, 82.40, 112.48, 122.40, 128.20 and 131.70-148.98 ppm for steroid

moiety; 47.20 and 55.00 ppm for methylene groups bound to both amino groups; at 50.22 and 82.36 ppm for oxazine ring; at 111.20, 118.40-120.78, 123.30, 128.40, 128.72, 131.66 and 151.64 ppm for naphthalene group. Finally, the presence of compound **6** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 512.30. Additionally, the compound **6** was prepared by the reaction of estradiol with the compound **3** in presence of formaldehyde. It is important to mention here that the yield was higher with the method A in comparison with method B. This phenomenon may possibly be due to reaction conditions.

The third stage was accomplished by the esterification of hydroxyl groups involved in the compound **6** for synthesis of **8**. Several methods have been used to prepare ester groups. Despite its wide scope, these protocols have several drawbacks such as low stability and use for hazardous reagents for their preparation [20,21]. In this study the compound **8** was synthesized by the reaction of **6** with chloroacetyl chloride using triethylamine as catalyst. It is important to mention that chlorobutanone was also formed in the chemical structure of **8** by the reaction of secondary amine with chloroacetyl chloride; this reaction is similar to other compounds with secondary amine [22].

^{1}H NMR spectrum of **8** shows signals at 0.82 ppm for methyl group bound to steroid nucleus; at 1.12-2.52, 4.80 and 6.72-6.80 ppm for steroid moiety; at 2.58 and 2.64 ppm for methylene groups bound to both amino groups; at 3.60 ppm for amino group; at 3.80 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 4.06-4.10 for methylene groups of both chloroacetic acid groups; at 4.30-4.40 and 5.00-5.10 ppm for oxazine ring; at 7.00-7.80 ppm for phenylgroups. ^{13}C NMR spectrum of **8** contains peaks at 14.30 ppm for methyl group bound to steroid nucleus; at 24.58-37.20, 44.00-44.50, 54.94, 84.54,118.66, 130.64 and 135.12-144.52 ppm for steroid moiety; at 40.56 and 40.80 ppm for methylene groups of both chloroacetic acid groups; at 44.88 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 47.30, 55.10 ppm for methylene groups bound to both amino groups; at 50.36 and 82.34 ppm for oxazine ring; at 111.20, 118.22, 120.72-128.80, 131.70 and 151.66 for phenyl groups; at 166.74 and 168.00 ppm for chloroacetic acid groups. Finally, the presence of compound **8** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 664.24.

On the other hand, the fourth stage was achieved by the reaction of **4** with chloroacetyl chloride to form the compound **9** using triethylamine as catalyst.In this reaction the hydroxyl group was esterified. However, a chloroamide group was also formed. It is important to mention that there are many procedures for the formation of chloroamides are known in the literature, for example the reaction of amine with trichloroisocyanuric acid [23] or secondary amide with *N*-chlorobenzotriazole to form a chloroamide derivative [24]. In addition, have been prepared some chloroamide groups using chloroacetyl chloride [25]. The results of ^{1}H NMR spectrum of **9** shows signals at 0.82 ppm for methyl group bound to steroid nucleus; at 1.12-2.52, 4.82 and 6.72-6.84 ppm for steroid moiety; at 2.66 and 3.38 for methylene groups bound to both amino groups; at 3.80 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 4.00 ppm for methylene group bound to amide group; at 4.08-4.12 ppm for both chloroacetic acid groups; at 5.76 ppm for both amino and amide groups. ^{13}C NMR spectrum of **9** contains peaks at 14.22 ppm for methyl group bound to steroid nucleus; at 24.58-37.20, 44.00-44.50, 50.82 and 84.56-144.36 ppm for steroid moiety; at 38.46 and 52.80 ppm for methylene groups bound to both amine groups; at 40.50 and 40.72 ppm for both chloroacetic acid groups; at 42.36 ppm for methylene bound to amide group; at 44.76 ppm for methylene group bound to both ring A of steroid nucleus and amino group; at 162.50 ppm for amide group; at 166.76-168.08 ppm for both ester groups. Finally, the presence of compound **9** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 572.16.

The fifth stage was accomplished by protecting the hydroxyl group of the compound **4**. It is important to mention that several triorganosilyl groups have been employed for protection of hydroxyl groups such as *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl [26]. In this study, the compound **4** was formed by reacting with *tert*-butyldimethylsilyl chloride to form the compound **10**. ^{1}H NMR spectrum of **10** shows signals at 0.08 and 0.88 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 0.25 and 0.96 ppm for methyl groups involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 0.80 ppm for methyl group bound to steroid nucleus; 1.01-2.08, 2.42-2.50, 3.50, 242-2.50 and 6.58-6.80 ppm for steroid moiety; at 2.20 ppm for both amino groups, at 2.62-2.76 ppm for methylene groups bound to both amine groups; at 3.62 ppm for methylene bound to both ring A of steroid nucleus and amine group. ^{13}C NMR spectrum of **9** contains peaks at 4.20, 18.44 and 25.70 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 4.50, 17.76 and 25.74 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 15.18 ppm for methyl group bound to steroid nucleus; at 25.30-25.54, 27.65-37.28, 43.70-44.48, 51.42 and 82.56-150.76 for steroid moiety; at 41.52 and 53.30 ppm for methylene groups bound to both amine groups; at 45.88 for methylene group bound to both ring A and amino groups. Finally, the presence of compound **10** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 572.40.

The last stage was achieved by reaction of **10** with chloroacetyl chloride to form the compound **11** using triethylamine as catalyst. ^{1}H NMR spectrum of **11** shows signals at 0.06 and 0.88 ppm for protons involved in the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 0.22 and 1.00 ppm for protons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.02-2.50, 3.50 and 6.50-6.80 ppm for steroid moiety; at 2.72 and 3.40 ppm for methylene groups bound to amine groups; at 3.64 ppm for methylene group bound to both ring A of steroid nucleus and amine group; at 4.00 ppm for chloroamidegroup; at 5.00 ppm for amino and amide groups. ^{13}C NMR spectrum of **11** contains peaks at 4.50, 17.76 and 25.74 ppm for carbons involved in

the *tert*-butyldimethylsilane fragment bound to ring D of steroid nucleus; at 4.20, 18.42 and 25.70 ppm for carbons involved in the *tert*-butyldimethylsilane fragment bound to ring A of steroid nucleus; at 15.18 ppm for methyl group bound to steroid nucleus; at 23.30-25.54, 27.60-37.28, 43.70-44.45, 51.49 and 82.60-150.78 ppm for steroid moiety; at 38.57 and 52.80 ppm for methylene groups bound to both amine groups: at 42.40 ppm for chloroamidegroup; at 45.83 ppm for methylene group bound to both ring A of steroid nucleus and amine group; at 162.56 for amide group. Finally, the presence of compound **11** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 648.36.

Biological activity: In order to evaluate the possibility of that compounds synthesized may have biological characteristics. In this study its antibacterial activity (minimal inhibitory concentration, MC) on Gram-negative (*Salmonella typhi*) bacteria was evaluated. The results showed (Figs. 6 and 7) that only the compounds **6** (MIC = 1.95×10^{-3} mmol), **9** (MIC = 1.74×10^{-3} mmol), **10** (MIC = 2.05×10^{-3} mmol) and **11** (MIC = 1.44×10^{-3} mmol) have antibacterial activity on *Salmonella typhi* in a dose manner dependent. Nevertheless, this effect was different in comparison with the controls (cefotaxime, MIC = 2.62×10^{-4} mmol; gentamycin, MIC = 1.29×10^{-4} mmol; and ciprofloxacin, MIC = 1.88×10^{-4} mmol). Analyzing these data, the antibacterial activity of a mixture of steroid derivatives was also evaluated using several systems (Fig. 7), The results showed that system G (mixture of all estrogen derivatives) exert higher antibacterial effect on *Salmonella typhi*. All these data indicate that antibacterial activity exerted by the estrogen derivatives on *Salmonella typhi* depend of their structure chemical in comparison with the controls and other steroid derivatives that are involved in this study. This phenomenon may involve the interaction of these compounds with some components of the bacterial cell, which may result in disturbance of bacterial growth and induce cell death, through perturbation of membrane bacterial. In this sense, the intramolecular interaction of compounds could be *via* divalent cations such as Mg^{2+} and Ca^{2+} involved in the membrane, consequently resulting a substantial increase the permeability of the outer membrane of *Salmonella typhi* as happening with other type of antibacterial agents [8-11,13].

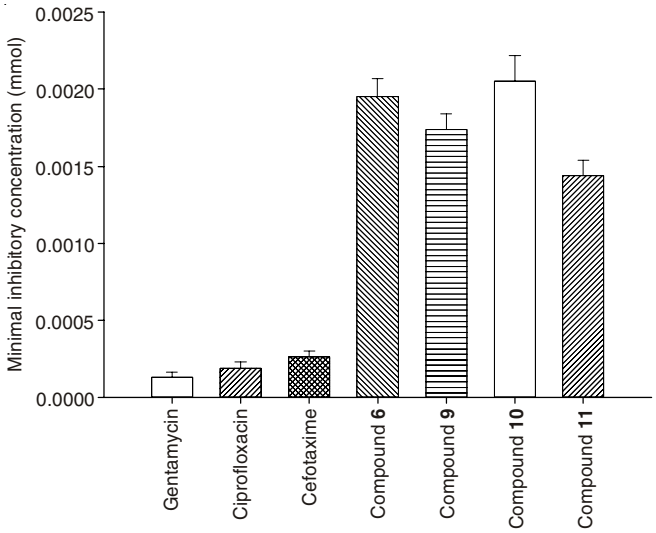


Fig. 6. Antibacterial activity induced by steroid derivatives (compound **6**, **9**, **10** and **11**) and controls (cefotaxime, CEFOT; gentamicin, GENT; and ciprofloxacin, CIPROF) on*Salmonella typhi*. Experimental data showed that *Salmonella typhi*was susceptibly to CEFOT (MIC = 2.62×10^{-4} mmol), GENT (MIC = 1.29×10^{-4} mmol) and CIPROF (MIC = 1.88×10^{-4} mmol). In addition, in presence of the compounds **6** (1.95×10^{-3} mmol), **9** (1.74×10^{-3} mmol), **10** (2.05×10^{-3} mmol) and **11**(1.44×10^{-3} mmol) the bacterial growth of this microorganism was inhibit. Each bar represents the mean ± S.E. of 9 experiments. MIC = Minimal inhibitory concentration

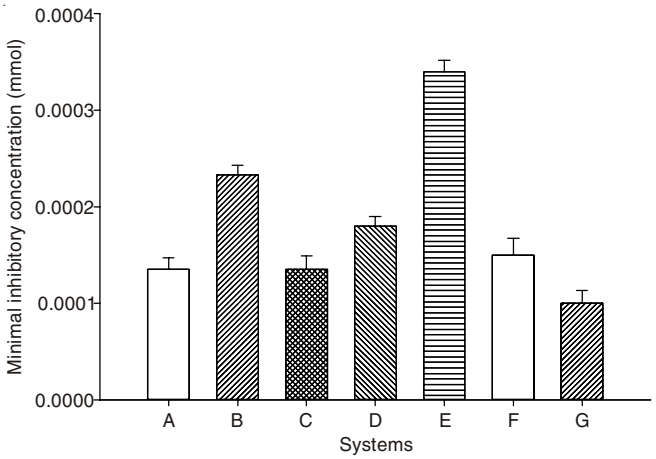


Fig. 7. Antibacterial activity induced by a steroid derivatives mixture on *Salmonella typhi*. Experimental data showed that *Salmonella typhi* was susceptibly to system A (compounds **6** and **9**; MIC = 1.35×10^{-4} mmol), system B (compounds **6** and **10**; MIC = 2.33×10^{-4} mmol), system C (compounds **6** and **11**; MIC = 1.35×10^{-4} mmol), system D (compounds **9** and **10**; MIC = 1.80×10^{-4} mmol), system E (compounds **9** and **11**; MIC = 3.40×10^{-4} mmol), system F (compounds **10** and **11**; MIC = 1.50×10^{-4} mmol), system G (compounds **6**, **9**, **10** and **11**; MIC = 1.20×10^{-4} mmol). Each bar represents the mean ± SE of 9 experiments. MIC = Minimal inhibitory concentration

Conclusion

In this study, new steroid derivatives with antibacterial activity on *Salmonella typhi* were synthesized using several strategies, which provide some advantages such as simple procedure and ease of workup in comparison with other techniques involved in the synthesis of other steroid derivatives.

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Theoretical analysis of biological activity of a new oxocin-steroid derivative against aromatase enzyme using a docking model

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Abstract
Several aromatase inhibitors have been prepared for treatment of breast cancer; however, the site of interaction with enzyme surface is not very clear. Therefore, the objective of this investigation was to synthesize and analyze the theoretical activity of a new oxocin-steroid derivative against aromatase (2dw3 protein) in a docking model using some aromatase antagonist (anastrozole and exemestane) as controls. In addition, physicochemical some parameters were determinate such as the inhibition constant (Ki). The results showed that only some of these aminoacid residues involved the surface of the 2dw3 protein may participate in the interaction with anastrozole, exemestane and compound 6. In addition, Ki value was low for exemestane compared with anastrozole and compound **6**; however, this physicochemical parameter was similar to both anastrozole and compound **6**. All these data suggest that compound **6** could be a good candidate as an aromatase inhibitor which translates as a possible drug for breast cancer.

Keywords: Steroid, Aromatase, Protein, Inhibition constant, Docking, Anastrozole, Exemestane.

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1. Introduction

Cancer breast is main cause of death in female the worldwide, which could be conditioned by several risk factors such as genetic, lifestyle, radiation, weigh, alcohol and others [1]. There are several studies which indicates that estrogen levels may predispose to development breast cancer in women [2-4]; some medicaments have been used for treatment of this clinical pathology such as estrogen-receptor inhibitors (tamoxifen and fulvestrant) [5, 6] However, several reports indicate that other drugs can exert their action as aromatase-enzyme inhibitors [7, 8]. For example, a report showed that treatment with letrozole (aromatase inhibitor) has beneficial effects against breast cancer in postmenopausal women previously treated with estrogen [9]. Other data showed that an aromatase inhibitor (anastrozole) exert cytotoxic effects against the MCF7 breast cancer cell line using a colorimetric test (MTT assay) [10]; however, some these drugs can produce several adverse effects [11]. In the search of other therapeutic alternatives, a series of drugs have prepared for treatment of breast cancer; for example, the synthesis of piperidine-2,6-dione derivative by the reaction of a phenylpiperidine-2,6-dione analog with sulfuric acid/nitric acid with biological activity against aromatase enzyme [12]. Other report showed the preparation of some aromatase inhibitors (imidazol-1-yl derivatives) from bromomethyl and imidazole using an *in vitro* model [13]. In addition, a steroid derivative (DTXSID70473247) was prepared from androstenedione via Clemmenson reaction and their biological activity on aromatase was evaluated using placental microsomes [14]. Also, other study showed the synthesis of pyridyl-tetralones derivatives through an aldol condensation of 1-tetralones with 4-pyridinecarboxaldehyde as human placental aromatase inhibitors [15]. Other report indicates the preparation and analyze of pharmacological activity of some imidazolyl-coumarins analogs as human placental aromatase inhibitors [16]. All these data indicate that several compounds can block the biological effect of aromatase; nevertheless, the interaction with enzyme surface is not very clear, so more studies are needed on this phenomenon. Analyzing, this hypothesis, in this study several estrone derivatives were synthesized and a theoretical analysis was carried out on their interaction with aromatase protein (2dw3) using a docking model.

2. Experimental

2.1 General methods

The compounds amino-estradiol (**1**) and amino-estrone (**2**) were synthesize using a previously method reported [17]. In addition, all the reagents used in this study were purchased from Sigma-Aldrich Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were determined using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{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.

2.2 Chemical Synthesis

Preparation of 2-(tert-Butyl-dimethyl-silanyloxy)-naphthalene-1-carbaldehyde (2).

In a round bottom flask (10 ml), 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol), terbuthyldimethylsilane chloride (200 µl, 1.07) chloroform (1 ml), were stirred to room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:bencene (4:1) system; yielding 37% of product; m.p. 98-100°C; IR (V_{max}, cm^{-1}) 1740 and 1112: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.20 (s, 6H), 0.96 (m, 9H), 7.02-9.22 (m, 6H), 10.14 (s, 1H, J = 0.58) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.24, 18.44, 25.72, 114.00, 114.52, 119.70, 124.40, 128.20, 128.62, 133.62, 135.20, 160.62, 190.74 ppm. EI-MS m/z: 286.13. Anal. Calcd. for $C_{17}H_{22}O_2Si$: C, 71.28; H, 7.74; O, 11.17; Si, 9.80. Found: C, 71.20; H, 7.70.

Synthesis of tert-Butyl-dimethyl-[1-(5,6,7,8-tetrahydro-2H-oxocin-2-yl)-naphthalen-2-yloxy]-silane (3)

In a round bottom flask (10 ml), compound **2** (200 mg, 0.70 mmol), 5-hexyn-1-ol (100 µl, 0.90 mmol) and Iron(III) chloride anhydrous (120 mg, 0.74 mmol) in 5 ml of methanol, were stirred to

room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:1) system; yielding 65% of product; m.p. 66-68°C; IR (V_{max}, cm^{-1}) 1600 and 1112: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.20 (s, 6H), 0.96 (s, 6H), 1.32-1.70 (m, 4H), 2.20-4.66 (m, 5H), 5.54 (d, 1H, J = 0.78 Hz), 5.74 (d, 1H, J = 0.12 Hz), 6.92-7.80 (m, 6H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: -4.24, 18.44, 25.24, 25.40, 25.70, 29.28, 67.30, 68.64, 113.72, 118.8 4, 124.22, 126.72, 126.80, 127.32, 127.92, 129.90, 131.94, 134.00, 135.25, 151.60 ppm. EI-MS m/z: 368.21. Anal. Calcd. for $C_{23}H_{32}O_2Si$: C, 74.95; H, 8.75; O, 8.68; Si, 7.62. Found: C, 74.90; H, 8.70.

1-[(3E)-5,6,7,8-tetrahydro-2H-oxocin-2-yl]naphthalen-2-ol (4).

In a round bottom flask (10 ml), compound **3** (200 mg, 0.54 mmol), hydrofluoric acid (1 ml), were stirred to room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:2) system; yielding 45% of product; m.p. 78-80°C; IR (V_{max}, cm^{-1}) 3380, 1602 and 1112: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.32 (m, 2H), 1.70 (m, 2H), 2.20-4.90 (m, 5H), 5.54 (d, 1H, J = 0.78 Hz), 5.74 (d, 1H, J = 0.12 Hz), 6.80 (broad, 1H), 7.14-7.66 (m, 5H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 25.24, 25.38, 29.32, 66.60, 68.62, 119.22, 119.32, 124.26, 126.36, 126.80, 127.40, 129.98, 130.12, 131.46, 132.72, 134.00, 150.92 ppm. EI-MS m/z: 254.13. Anal. Calcd. for $C_{17}H_{18}O_2$: C, 80.28; H, 7.13; O, 12.58. Found: C, 80.22; H, 7.12.

Preparation of (11aS)-11a-methyl-8-({1-[(3E)-5,6,7,8-tetrahydro-2H-oxocin-2-yl]naph-thalen-2-yl}oxy)-1H,2H,3H,3aH,3bH,4H,5H,9bH,10H,11H-cyclopenta[a]phenan- threne-1,7-diol (5)

In a round bottom flask (10 ml), compound **4** (200 mg, 0.79 mmol), 2-nitroestradiol (200 mg, 0.63 mmol) and potassium carbonate (100 mg, 0.72 mmol) in 5 ml of dimethyl sulfoxide were stirred to room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:2) system; yielding 38% of product; m.p. 104-106°C; IR (V_{max}, cm^{-1}) 3400, 1600 and 1110: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-1.14 (m, 4H), 1.30 (m, 2H), 1.33-1.66 (m, 4H), 1.70 (m, 2H), 1.76-2.10 (m, 2H), 2.20-2.26 (m, 2H), 2.46-3.64 (m, 4H), 3.86-4.28 (m, 3H), 5.56 (d, 1H, J = 0.78 Hz), 5.66 (d. 1H, J = 0.12), 5.90 (broad, 2H), 6.24-6.66 (m, 2H), 7.22-7.92 (m, 6H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 15.80, 24.22, 25.22, 25.34, 25.36, 27.76, 29.28, 29.66, 32.78, 33.71, 37.28, 44.00, 44.40, 50.74, 68.10, 68.62, 82.44, 114.43, 115.70, 118.80, 119.98, 122.47, 124.26, 126.82, 126.84, 128.01, 130.52, 130.84, 132.22, 133.50, 134.00, 136.14, 137.70, 145.67, 146.75 ppm. EI-MS m/z: 524.29. Anal. Calcd. for $C_{35}H_{40}O_4$: C, 80.12; H, 7.68; O, 12.20. Found: C, 80.08; H, 7.62.

7-hydroxy-11a-methyl-8-({1-[(3Z)-5,6,7,8-tetrahydro-2H-oxocin-2-yl]naphthalen-2-yl} oxy)-2H,3H,3aH,3bH,4H,5H,9bH,10H,11H-cyclopenta[a]phenanthren-1-one (6).

In a round bottom flask (10 ml), compound **4** (200 mg, 0.79 mmol), 2-nitroestrone (200 mg, 0.63 mmol) and potassium carbonate (100 mg, 0.72 mmol) in 5 ml of dimethyl sulfoxide were stirred to room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:2) system; yielding 52% of product; m.p. 120-122°C; IR (V_{max}, cm^{-1}) 3402, 1712, 1602 and 1112: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.92 (s, 3H), 1.20-1.28 (m, 3H), 1.30 (m, 2H), 1.33-1.54 (m, 2H), 1.70 (m, 2H), 1.78-2.20 (m, 2H), 2.22-2.26 (m, 2H), 2.46-2.80 (m, 4H), 3.86-4.28 (m, 3H), 5.36 (broad, 1H), 5.56 (d, 1H, J = 0.78 Hz), 5.66 (d. 1H, J = 0.12), 6.32-6.68 (m, 2H), 7.24-7.92 (m, 6H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.80, 21.76, 25.22, 25.38, 25.86, 26.44, 29.26, 29.64, 31.51, 35.43, 37.56, 46.87, 48.10, 50.40, 68.10, 68.62, 114.42,115.34, 118.80, 120.00, 122.47, 124.26, 126.82, 126.84, 127.64, 130.53, 130.84, 132.20, 12.92, 133.50, 134.00, 135.64, 137.72, 145.64, 146.74, 220.70, ppm. EI-MS m/z: 522.27. Anal. Calcd. for $C_{35}H_{38}O_4$: C, 80.43; H, 7.33; O, 12.24. Found: C, 80.36; H, 7.27.

2.3 Physicochemical Parameters Evaluation

The parameters hydrogen bond acceptor (HBA), hydrogen bond donator (HBD), topological polar surface area (TPSA) and partition coefficient (cLog) of compound 5, 6, anastrozole and exemestane were evaluated using LigandScout software 4.3 [18].

2.4 Theoretical evaluation of the interaction between compounds 3 or 7 with aromatase

Theoretical analysis of interaction of compounds 2-7 on aromatase protein (2dw3) [19] was carried out using a docking program (DockingServer) [20]. In addition, two aromatase inhibitors (anastrozole, exemestane) [21, 22] were used as controls.

3. Results and Discussion

Some compounds have synthesized as aromatase inhibitors [7-10], nevertheless, the site of interaction with enzyme surface is not very clear, so more studies are needed on this phenomenon. Therefore, the aim of this study, some steroid derivatives was synthesized to evaluate their theoretical interaction with aromatase enzyme using a Docking model [18, 20].

3.1 Protection of hydroxyl group

The first stage was achieved protecting the hydroxyl group of the 2-Hydroxy-naphthalene-1-carbaldehyde in order to avoid possible reaction of hydroxyl group with any substance involved in the following reaction. It is important to mention that several organosilyl groups have been employed for protection of hydroxyl groups such as tert-butyldimethylsilyl and tert-butyldiphenylsilyl [23].

In this study, the 2-Hydroxy-naphthalene-1-carbaldehyde reacted with tert-butyldimethylsilyl chloride, Figure (1) to form the compound **2** (2-(tert-Butyl-dimethyl-silanyloxy)-naphthalene-1-carbaldehyde). The ^{1}H NMR spectra for **2** showed several signals at 0.20-0.96 ppm for terbuthyldimethylsilane fragment; at 7.02-9.22 ppm for ohenyl groups; at 10.14 ppm for aldehyde group. ^{13}C NMR spectrum for **2** showed some signals at -4.24-25.72 for terbuthyldimethylsilane fragment; at 114.00-160.62 ppm for phenyl groups; at 190.74 ppm for aldehyde group. In addition, the mass spectrum from **2** showed a molecular ion (m/z) at 286.13.

3.2 Preparation of a tetrahydro-2H-oxocine ring

There are some reports which indicate the synthesis of tetrahydro-2H-oxocine rings using several reagents such as benzylidene-bis(tricyclohexylphosphino)-dichlororuthenium [24], palladium derivative [25], vanadium-haloperoxidase [26], azobisisobutyronitrile/*p*-Toluene-sulfonylbro- mide [27] and others. In this reaction, **2** was reacted with Iron(III) chloride to form a tetrahydro-2H-oxocine ring involved in the chemical structure of the compound **3**, Figure (1). The ^{1}H NMR spectra for **3** showed some chemical bands at 0.20-0.96 ppm for terbuthyldimethylsilane fragment; at 1.32-5.74 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 6.92-7.80 ppm for phenyl groups. ^{13}C NMR spectrum for **3** showed some signals at -4.24-18.44 and 25.70 ppm for terbuthyldimethylsilane fragment; at 25.24-25.50, 29.28-68.62, 126.80 and 134.00 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 113.72-126.72, 127.32-131.94 and 135.25-151.60 ppm for phenyl groups. Finally, the mass spectrum from **3** showed a molecular ion (m/z) at 368.21.

3.3 Removal of Silane group

Some reagents have been used to removal silyl protecting groups from hydroxyl such as ammonium fluoride [28], tris(dimethylamino)sulfonium/difluo-rotrimethyl silicate [29], hydrofluoric acid [30] and others. In this study, hydrofluoric acid was used to removal of silyl-protecting group from hydroxyl of the compound **3** to form **4**, Figure (1). ^{1}H NMR spectra for **4** showed several signals at 1.32-5.74 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 6.80 ppm for hydroxyl group; at 7.14-7.66 ppm for phenyl group. ^{13}C NMR spectrum for **4** showed some signals at 25.40-68.62, 126.80 and 134.00 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 119.22-126.36, 127.40-132.72 and 150.92 ppm for phenyl groups. Additionally, the mass spectrum from **4** showed a molecular ion (m/z) at 254.13.

3.4 Esterification of 2-nitroestradiol or 2-nitroestrone

It is noteworthy that there are many procedures for preparation of several ether derivatives; however, despite its broad scope, they have some drawbacks; For example, several reagents used are hazardous and expensive such as Iodophenol, 1,4-diazabicy- clo[2.2.2]octane, 2,2,6,6-tetramethylheptane-3,5-dio- ne aryl-trifluoroborate salts [31]. Another data indicates that formation of ether groups via displacement of nitro groups with hydroxyl groups using a dipolar aprotic solvent; In general, dipolar solvents are used to attain high yield of ether groups [32]. In this study, the compound **4** was reacted with 2-nitro estradiol or 2-nitroestrone in presence of dimethyl sulfoxide at mild conditions, Figure (2) to form two ether derivatives (compound **5** or **6**).

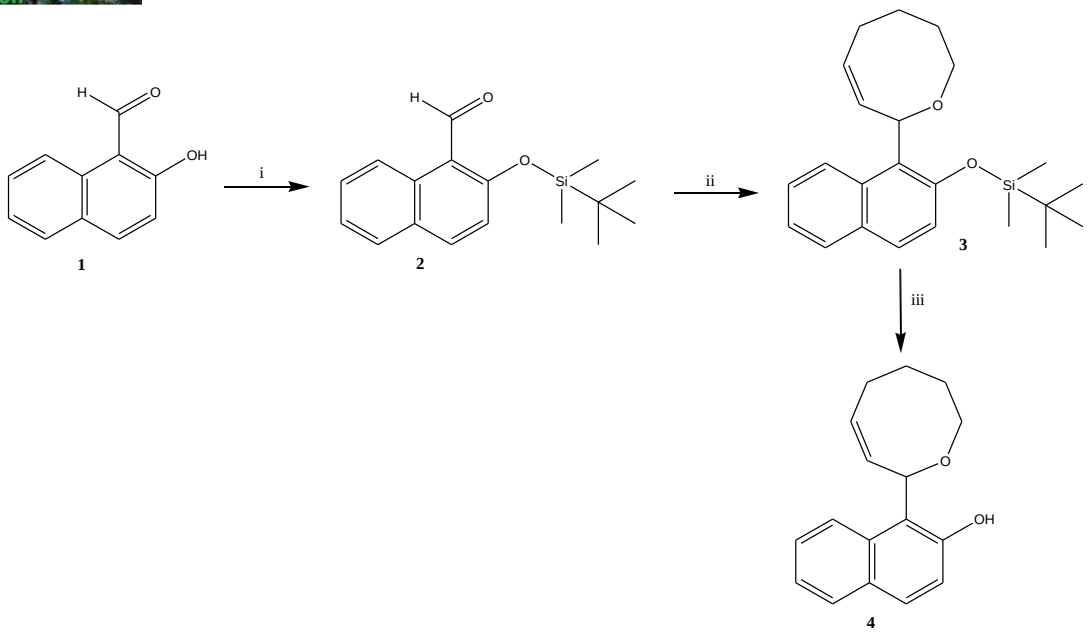

Figure 1. Synthesis of an oxocin-naphthalen-2-ol derivative (**4**). Reaction of 2-hydroxy-1-naphthaldehyde (**1**) with terbuthyldimethyl-sylane chloride (i) to form the 2-(tert-Butyl-dimethyl-silanyloxy)-naphthalene-1-carbaldehyde (**2**). Then **2** was reacted with 5-hexyn-1-ol (ii) to synthesis of the tertbtyldimethyllilane-oxocin-naphthalen analog (**3**). Finally, **4** was prepared by the reaction of 3 with hydrofluoric acid (iii).

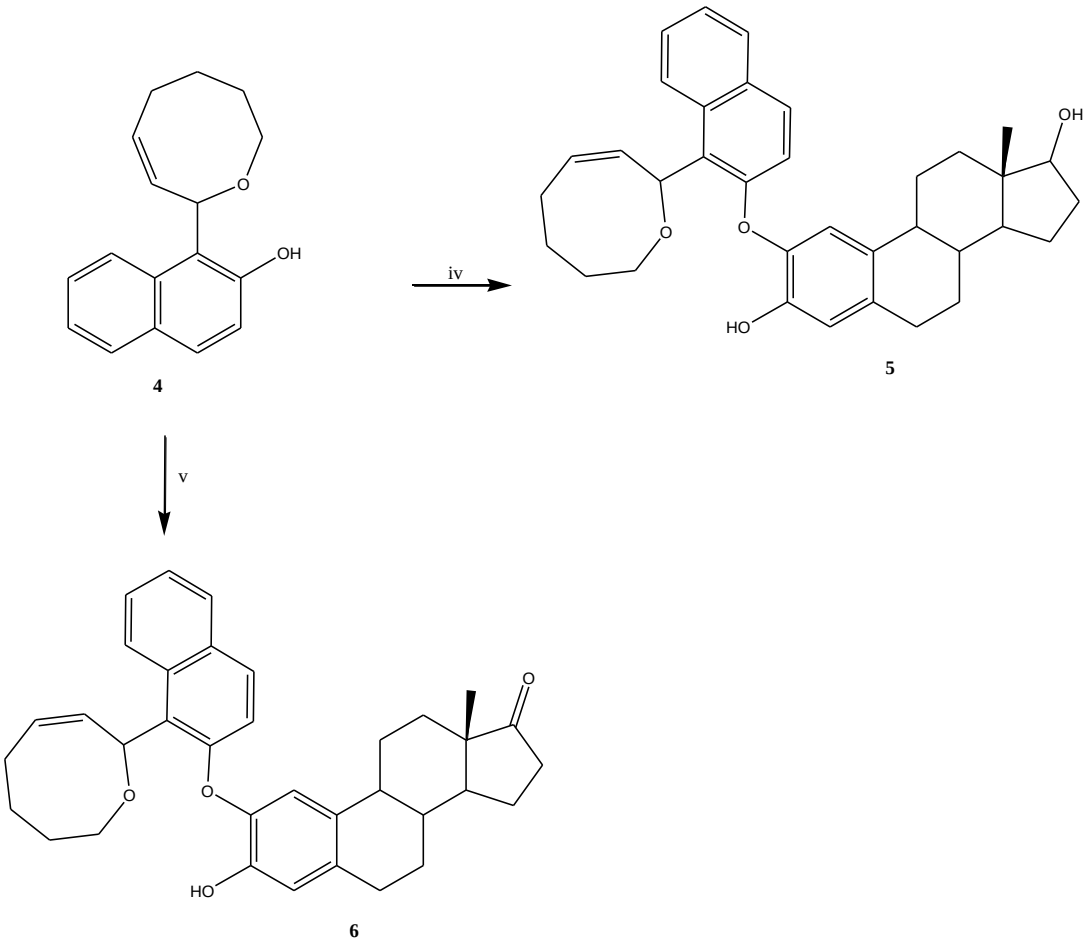

Figure 2. Preparation of two oxin-steroid derivatives (**5** or **6**). Reaction of oxocin-naphthalen-2-ol derivative (**4**) with 2-nitroestradiol (iv) or 2-nitroestrone (v) to form the oxin-naphalen-cyclopenta[a]phenanthrene-1,7-diol (**5**) or oxin-naphalen-cyclopenta[a]phenanthrene-1-one (**6**).

^{1}H NMR spectra for **5,** Figure (2) showed several signals at 0.76 for methyl group bound to steroid nucleus; at 0.80-1.14, 1.33-1.66, 1.76-2.10, 2.46-3.64 and 5.24-6.66 ppm for steroid moiety; at 1.30, 1.70, 2.20-2.26 and 3.86-5.66 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 7.22-7.92 ppm for phenyl groups; at 5.88 for hydroxyl group. ^{13}C NMR spectrum for **5** showed some signals at 15.80 ppm for methyl group bound to steroid nucleus; at 24.22. 25.34, 27.76, 29.66-50.74, 82.44-115.70, 128.00, 136.14 and 145.67-146.75 ppm for steroid moiety; at 25.22, 25.36, 29.80, 68.10-68.62, 126.84 and 134.00 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 118.80-126.82, 130.52-133.50 and 137.70 ppm for phenyl groups. In addition, the mass spectrum from **5** showed a molecular ion (m/z) at 524.29.

Finally other results showed several signals of ^{1}H NMR spectra for **6**, Figure (2) at 0.92 for methyl group bound to steroid nucleus; at 1.20-1.28, 1.33-1.54, 1.78-2.20, 2.46-2.80 and 6.32-6.68 pm for steroid moiety; at 1.30, 1.70, 2.22-2.26, 3.86-4.28 and 5.56-5.66 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 5.36 ppm for hydroxyl group; at 7.24-7.92 ppm for phenyl groups.

^{13}C NMR spectrum for **6** showed some signals at 13.80 ppm for methyl group bound to steroid nucleus; at 26.76, 25.86-26.44, 29.64-50.40, 114.42-115.34, 127.64, 135.64 and 145.64-146.74 ppm for steroid moiety, at25.22-25.38, 29.26, 68.10-68.62, 126.04 and 134.00 ppm for 3,4,5,8-Tetrahydro-2H-oxocine ring; at 118.80-126.82, 130.53-133.50 and 137.72 ppm for phenyl group; at 220.70 ppm for ketone group. Finally, the mass spectrum from **6** showed a molecular ion (m/z) at 522.27.

3.5 Theoretical analysis

Interactions between molecule-protein and protein-protein are involved in several biological processes such as signal transduction, physiological regulation, gene transcription, and enzymatic reactions [33]. It is important to mention that several drugs can induce changes biological activity of some biological system via interactions with either specific protein or enzyme; therefore, several theoretical models have been developed to predict the interaction of drugs with different proteins or enzymes [34]. In this sense, in this study some physicochemical factors involved in the interaction of compounds 5 or 6 with aromatase were evaluated using as control to anastrozole and exemestane (aromatase inhibitors).

3.6 Physicochemical parameters evaluation

There are several structure-activity studies which suggest that some physicochemical factors are involved in the activity of several drugs, such as hydrogen bond donor groups. (HBD) and hydrogen bond acceptor groups (HBA) may exert also changes on some biological system [35]. In this regard, these physicochemical descriptors have been evaluated using some pharmacophore models [36, 37]; It is important to mention that pharmacophores are generally used to evaluate some chemical characteristics that are related with the biological activity of several molecules; therefore, in this study a theoretical study was carried out using a pharmacophore model [38]. Therefore, these physicochemical factors involved in the chemical structure of anastrozole, exemestane and compounds **5** or **6** were asses, Figures (5) and (6).

The theoretical results showed several hydrogen bond acceptor groups for anastrozole (both nitrogen atom and cyanide group); for exemestane (carbonyl group); for compound **5** (ether group); for compound **6** (both carbonyl and ether groups). other results shown some hydrogen bond acceptor groups for exemestane (cyanide group); for compound **5** (hydroxyl group); for compound **6** (hydroxyl groups). In addition, the theoretical results, Table (1) showed both HBA (< 10) and for HBD (< 5) values for compounds **5** and **6**. Analyzing these results and other reports about Lipinski's rule which indicates that both HBD and HBA can condition some pharmacokinetic process of drugs in the human body [39]; these data suggest that compounds **5** or **6** could have the ability of penetrate some barrier biological of human body.

However, it is noteworthy that the rule does not predict if a compound could be pharmacologically active; therefore, other type of studies must be carried out to determine the interaction between some compounds with several biological targets such as proteins or enzymes.

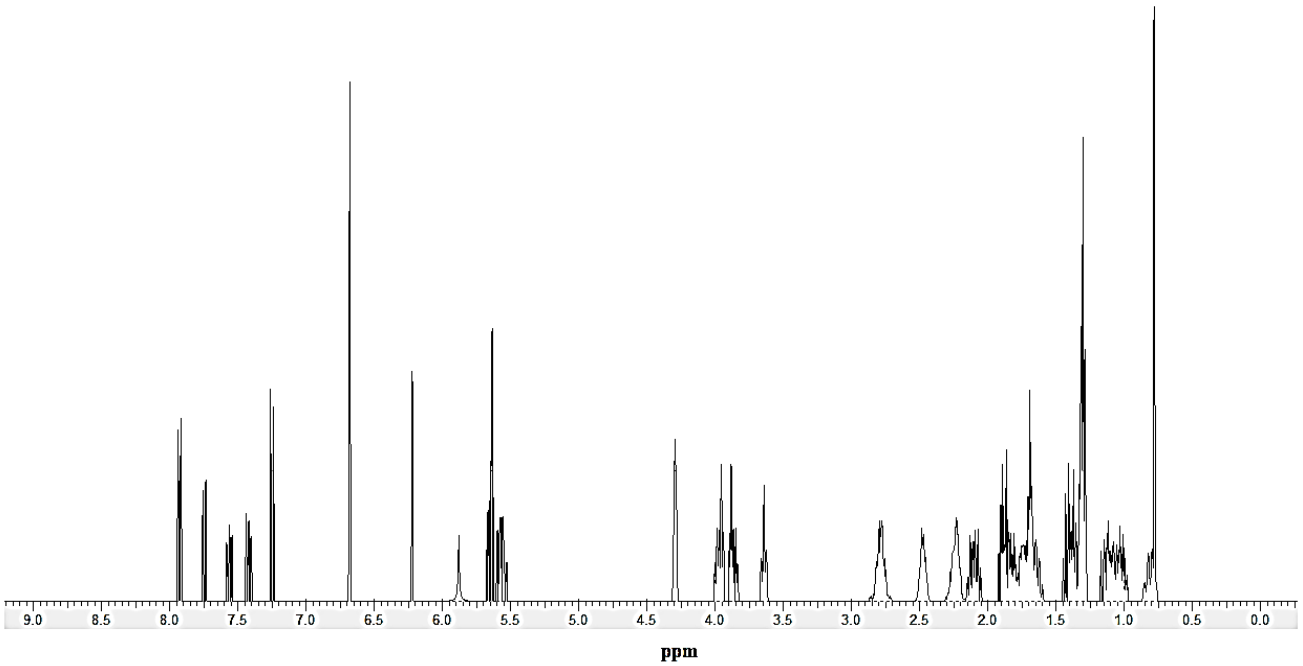

Figure 3. ^{1}H NMR spectrum of **5** was determinate with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in $CDCl_3$. ppm = parts per million.

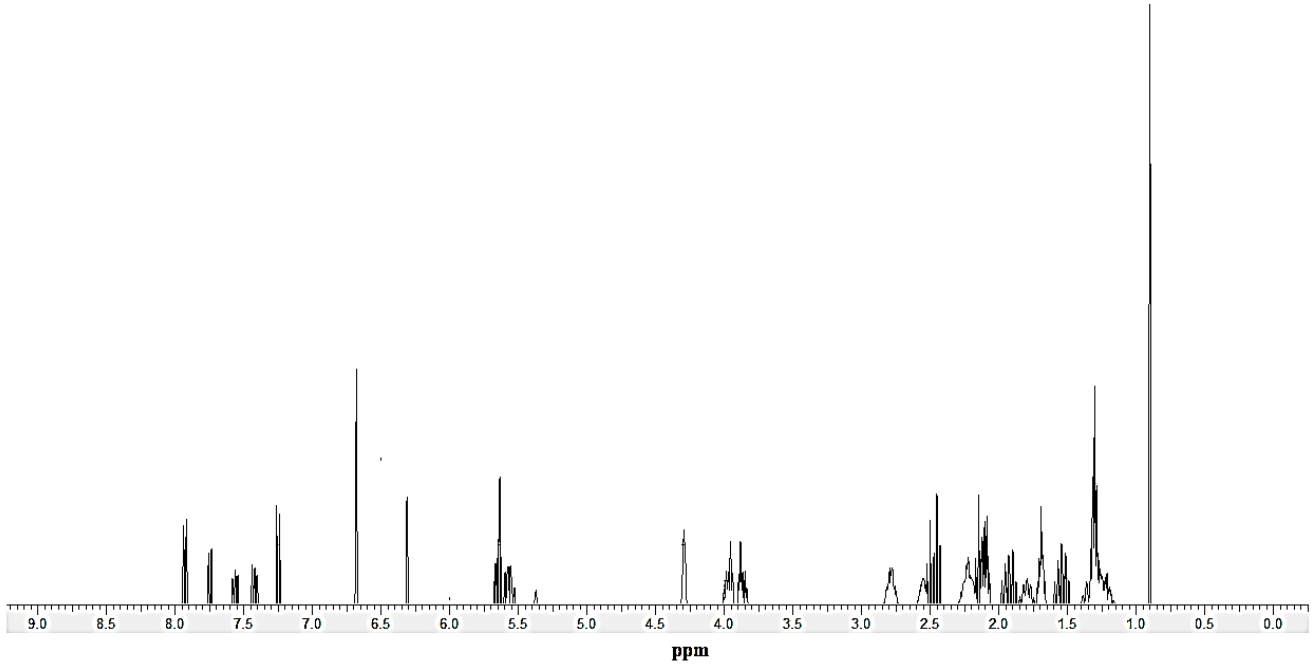

Figure 4. The scheme shown ^{1}H NMR spectrum of compound **6**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in $CDCl_3$. ppm = parts per million.

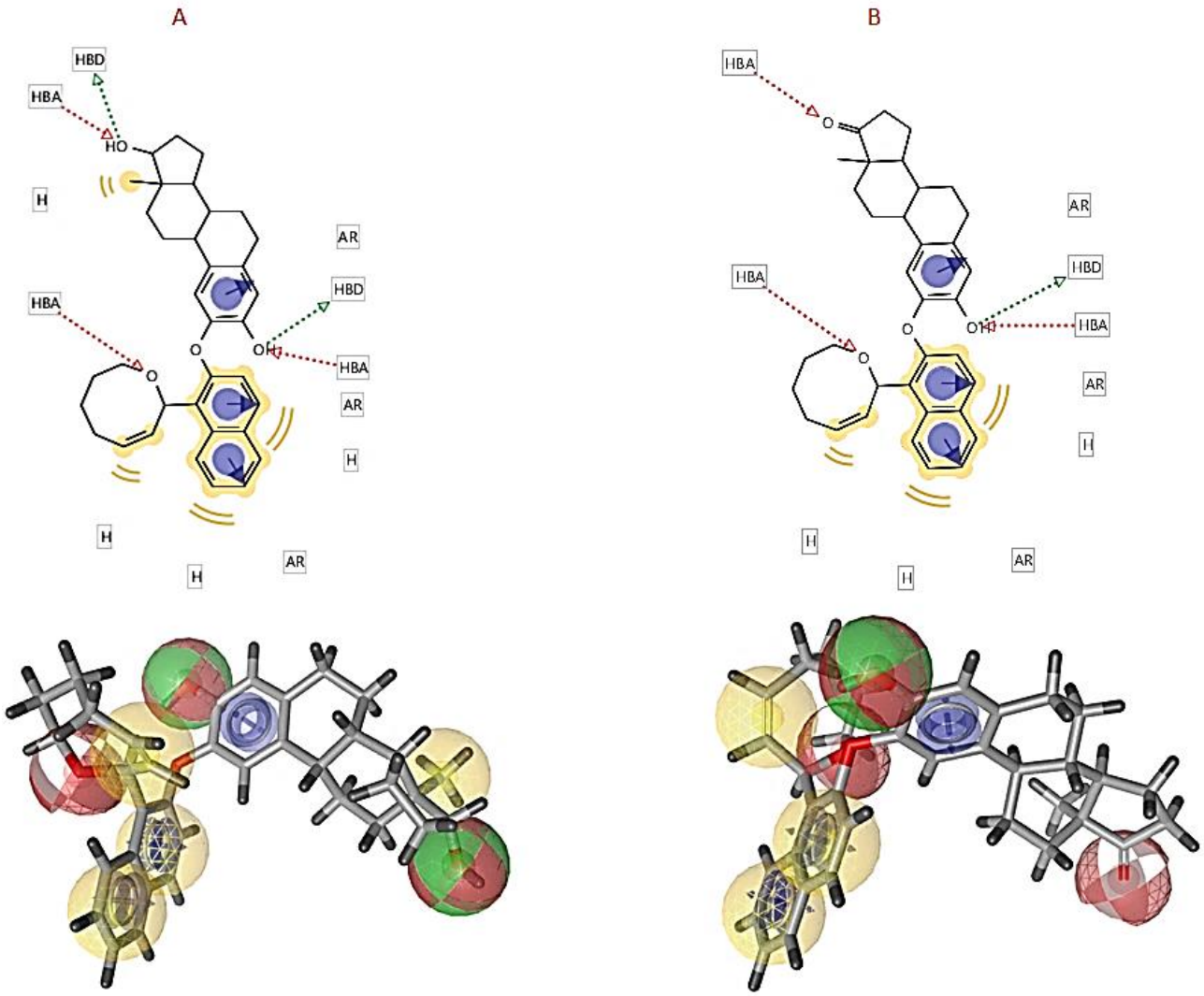

Figure 5. Scheme represents a pharmacophore from both compounds **5** (A) and **6** (B) using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

Table 1. Physicochemical parameters involved in the chemical structure of Anastrozole, Exemestane and Compounds **5** or **6**.

Parameter	Anastrozole	Exemestane	Comp. 5	Comp. 6
Rotable	8	2	6	5
cLog	2.79	4.03	8.06	8.27
TPSA	78.29	34.14	58.92	55.76
HBA	4	2	3	3
HBD	0	0	2	1

Hydrogen bond acceptor (HBA); hydrogen bond donator (HBD); topological polar surface area (TPSA); partition coefficient (cLog).

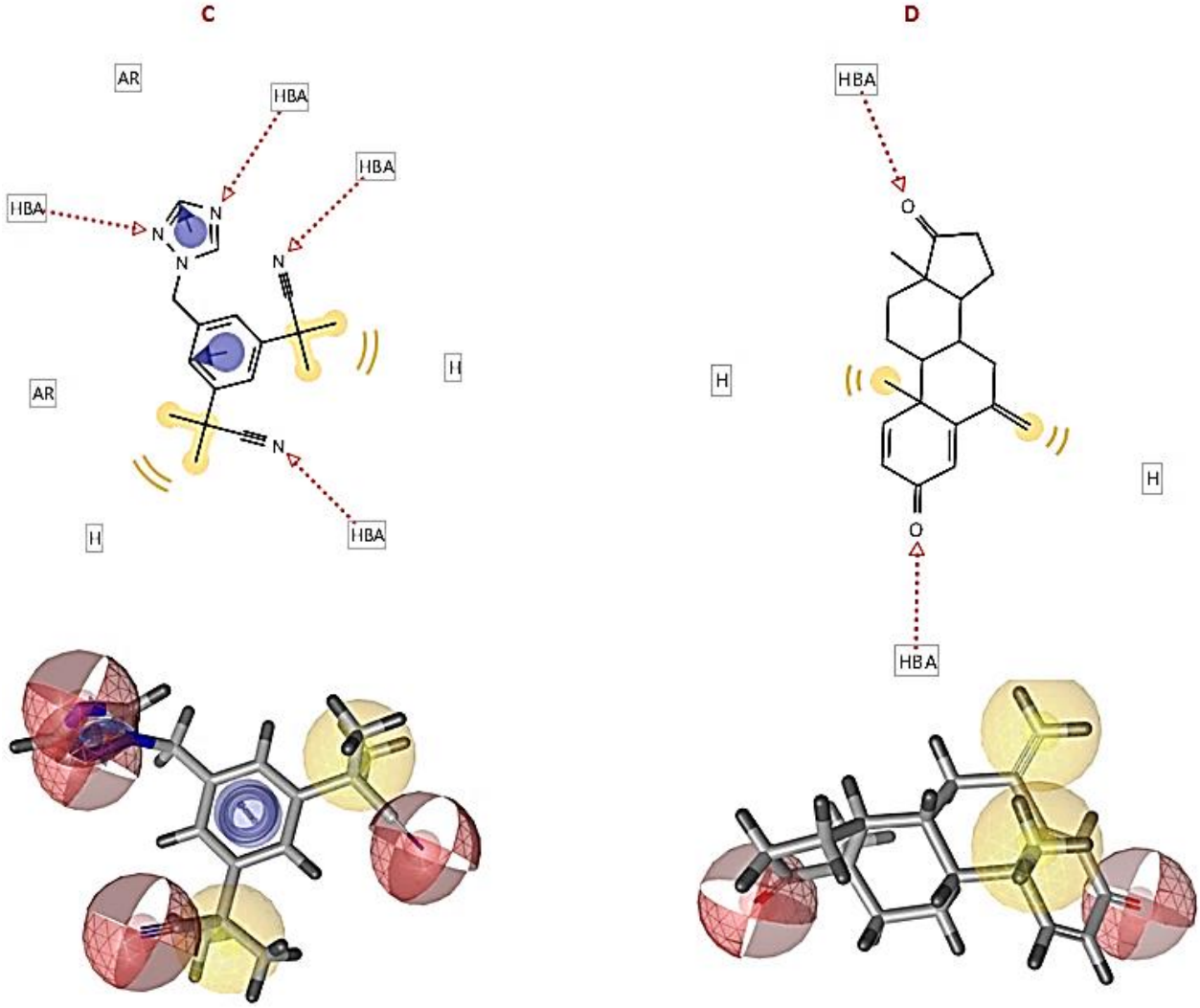

Figure 6. Pharmacophore from both anastrozole (C) and exemestane (D) using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

3.7 Theoretical analysis of interaction of compounds 3-7 with aromatase protein

Analyzing the data above mentioned, in this study was carried out a theoretical analysis on interaction of compounds **5** or **6** with aromatase protein (2wd3) [19] using a Docking model [20].

The results shown in Figures (7), (8) and Table (2) shown the interaction of compounds **5** or **6** with different type of aminoacid residues involved in enzyme surface (2wd3). To determine whether the compounds **5** or **6** could act as aromatase inhibitors; also, theoretical interaction of enzyme with some aromatase antagonists, such as anastrozole, and exemestane was evaluated. The results the Figures (7) and (8) and the Table (2) showed that compounds **5** or **6** could interact in a similar way with some amino acid residues compared to anastrozole and exemestane; this phenomenon could be due to different energy levels that are produced between each of intramolecular interactions.

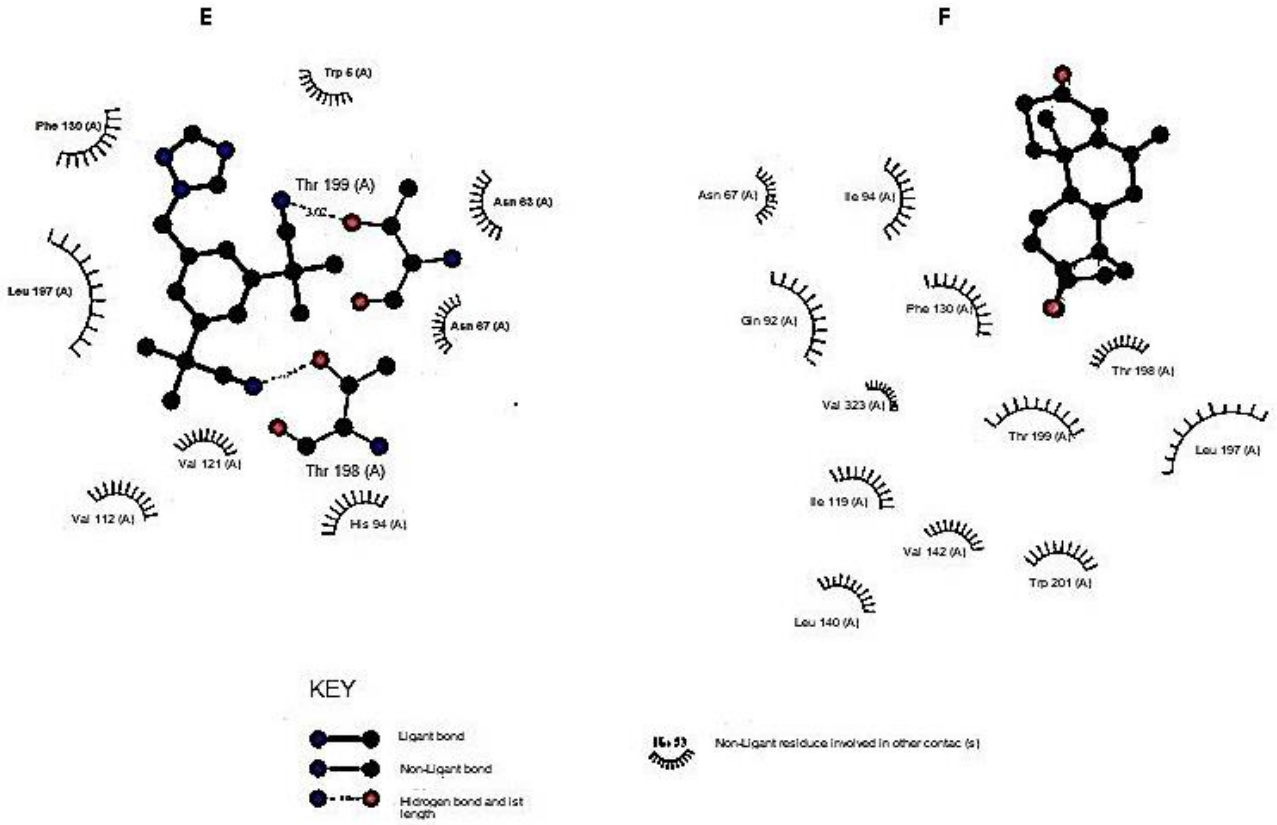

Figure 7. The scheme shows the binding of both anastrozole (E) and exemestane (F) with some aminoacid residues of the aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

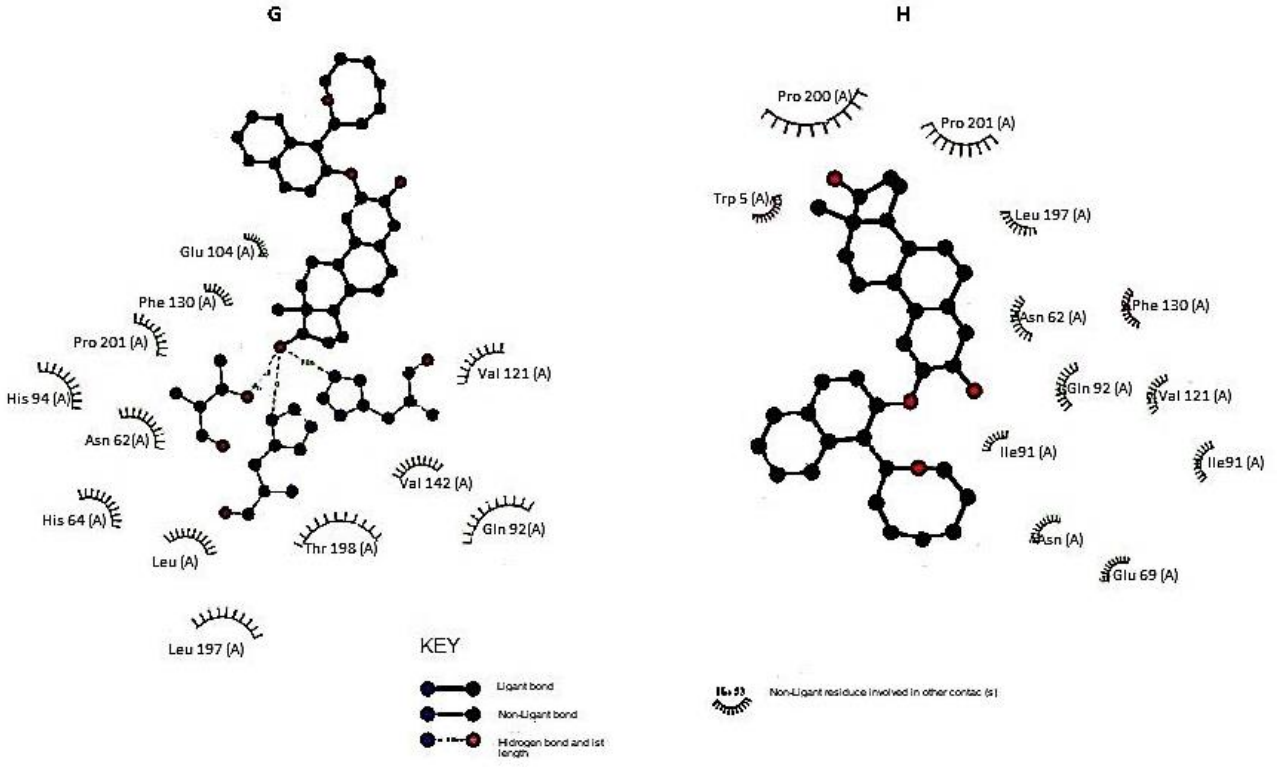

Figure 8. The scheme shows the binding of both compounds **5** (G) and **6** (H) with some aminoacid residues of the aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

Table 2. Residues aminoacids involved in the interaction between Anastrozole, Exemestane and Compounds **5** or **6** with 2dw3 protein.

Anastrozole	Exemestane	Comp. **5**	Comp. **6**
Trp_{5}	Asn_{67}	Trp_{5}	Trp_{5}
Asn_{62}	Gln_{92}	Asn_{62}	Asn_{62}
Asn_{67}	His_{94}	His_{64}	Asn_{67}
His_{94}	His_{119}	Gln_{92}	Glu_{69}
Val_{121}	Val_{121}	His_{94}	Ile_{91}
Phe_{130}	Phe_{130}	Glu_{104}	Gln_{92}
Val_{142}	Leu_{140}	His_{119}	His_{94}
Leu_{197}	Val_{142}	Val_{121}	Val_{121}
Thr_{198}	Leu_{197}	Phe_{130}	Phe_{130}
Thr_{199}	Thr_{198}	Val_{142}	Leu_{197}
	Thr_{199}	Leu_{197}	Pro_{200}
	Tpr_{208}	Thr_{198}	Pro_{201}
		Thr_{199}	
		Pro_{201}	

Red = similar aminoacid residues of anastrozole, exemestane and compounds 5 or 6; Blue = similar aminoacid residues of exemestane and compounds 5 or 6.

3.8 Thermodynamic parameters

Analyzing data above mentioned and some reports which indicate that several thermodynamic factors may be involved in the interaction drug-protein [40]; in this study, a theoretical ass was carried out on some thermodynamic parameters involved in the interaction of anastrozole, exemestane and the compounds **5** or **6** with the aromatase (2wd3 protein) such as 1) free energy of binding which determinate the energy value that require a molecule to interact with a protein in a water environment; 2) electrostatic energy that is the product of electrical charge and electrostatic potential, which are involved in the ligand-protein system [41]; 3) total intermolecular energy and 4) Van der Waals (vdW) + hydrogen bond (Hbond) + desolvation energy (Desolv. Energy); which have an influence on the movement of water molecules into or out of the ligand-protein system) [41] using a theoretical model (dockingserver) [20].

The results showed in the Table (3) indicate that all thermodynamic parameters were different for compounds **5** or **6** compared with anastrozole and exemestane.

This phenomenon indicates that there are differences in the energy levels between the interaction of the compounds studied and the 2wd3 protein, which can be translated as changes in the biological activity of aromatase in the presence of compounds **5 or 6** compared with anastrozole and exemestane. In addition, the inhibition constant (Ki) value was low for exemestane in comparison with the compounds **5** or **6**; however, Ki of anastrozole was in a similar manner to the compound **6**.

4. Conclusions

In this study, is reported a facile synthesis of two steroid derivative using some chemical strategies. In addition, the theoretical data suggest that compound **6** could be a good candidate to inhibit the biological activity of aromatase; however, it is important to mention to evaluate this hypothesis, several experiments must be carried out in some biological model.

Table 3. Thermodynamic parameters involved in the interaction of anastrozole, letrozole, exemestane and compounds **2-7** with aromatase (2dw3).

Parameter	Anastrozole	Exemestane	Comp. **5**	Comp. **6**
Est. Free Energy of Binding [kcal/mol]	-5.54	-6.82	-8.93	-9.46
Est. Inhibition Constant, Ki (μM)	86.91	10.00	282.93	86.87
vdW + Hbond + desolv. Energy [kcal/mol]	-7.35	-6.82	-9.74	-9.58
Electrostatic Energy [kcal/mol]	-0.02	-0.00	-0.10	-0.01
Total Intermolecular. Energy [kcal/mol]	-7.37	-6.82	-9.84	-9.57
Interact. Surface	683.29	671.15	926.16	912.45

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Preparation of five estrone analogs and theoretical analysis of its interaction with aromatase enzyme

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ABSTRACT

Several aromatase inhibitors have been prepared for treatment of breast cancer; nevertheless, their interaction with enzyme surface is not very clear. Therefore, the objective of this investigation was to synthesize and analyze the theoretical activity of five estrone derivatives (compounds **2**-**7**) on aromatase (4kq8 protein) in a theoretical method using some aromatase antagonist (anastrozole, letrozole and exemestane) as controls. The data found showed that both anastrozole and compound **6** could interact with same aminoacid residues such as Ile_{133}, Phe_{134}, Phe_{221}, Ala_{306}, Asp_{309}, Thr_{310}, Val_{310}, Val_{373}, Met_{374}, Leu_{477} and Ser_{478} that are involved in the 4kq8 protein surface. It is noteworthy that several of these aminoacid residues may be involved in the interaction between 4kq8 protein with compounds **2**-**5** and **7**, these differences could induce significantly changes in the biological activity of aromatase through of interaction with **6** compared with the compounds **2**-**5** and **7**. These results indicate that compound **6** could be a good candidate as an aromatase inhibitor which translates as a possible drug for breast cancer.

Keywords: *Estrone derivatives, breast cancer, aromatase, docking.*

1. INTRODUCTION

Cancer breast is main cause of death in female the worldwide, which could be conditioned by several clinical parameters such as genetic, lifestyle, radiation, weigh, alcohol and others [1]. In addition, some reports have been shown that estrogen levels may predispose to develop breast cancer in women [2-4]; it is noteworthy, that some medicaments are used to breast cancer such as estrogen-receptor inhibitors (tamoxifen and fulvestrant) [5, 6] or aromatase inhibitors (anastrozole, letrozol and exametane) [7]; nevertheless, several drugs can produce some adverse effects [8, 9]. Therefore, a series of drugs have prepared for treatment of breast cancer; for example, the synthesis of piperidine-2,6-dione derivative by the reaction of a phenylpiperidine-2,6-dione analog with sulfuric acid/nitric acid with biological activity against aromatase enzyme [10]. Other report showed the preparation of some aromatase inhibitors (imidazol-1-yl derivatives) from bromomethyl and imidazole using an *in vitro* model [11]. In addition, a steroid derivative (DTXSID70473247) was prepared from androstenedione via Clemmenson reaction and their biological activity on aromatase was evaluated using placental microsomes [12]. Also, a study shown the preparation of pyridyl-tetralones derivatives through an aldol condensation of 1-tetralones with 4-pyridinecarboxaldehyde as human placental aromatase inhibitors [13]. Other report indicates the preparation and analyze of pharmacological activity of some imidazolyl-coumarins analogs as human placental aromatase inhibitors [14]. These reports suggest that some drugs can block the biological effect of aromatase; nevertheless, their interaction with enzyme surface is very confusing. Therefore, the aim of this study was carried out the synthesis of several estrone derivatives to evaluate their interaction with the aromatase protein (4kq8) using a docking model.

2. EXPERIMENTAL SECTION

Chemical synthesis.

Both 2-nitroestrone and estrone-indole were prepared using previously methods reported [15, 16]. Additionally, other reagents involved in this study were purchased from Sigma-Aldrich Sigma-Aldrich Co., Ltd. The melting point of compounds was assessed using an Electrothermal (900 model). Infrared spectrum (IR) was evaluated using potassium bromide with a Perkin Elmer Lambda 40 apparatus. ^{1}H and ^{13}C NMR spectrum was analyzed on a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz (megahertz) in $CDCl_3$ (deuterated chloroform) using TMS as an internal standard. EIMS spectrum was obtained with a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis was determined using a Perkin Elmer Ser. II CHNS/02400 apparatus.

Preparation of 2-ntro-steroid*-indol-4-ol *derivative

Method A:

In a round bottom flask (10 ml), 2-nitroestrone (200 mg, 0.63 mmol), phenylhydrazine hydrochloride (100 mg; 0.69 mmol), and 8 ml of acetic acid:ethanol (3:5) were stirring to reflux for 4 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:1) system.

(8aS)-8a-methyl-5-nitro-1,2,6b,7,8,8a,9,14,14a,14b-decahydro-naphtho[2',1':4,5]indeno[1,2-b]indol-4-ol (3)

yielding 54 % of **3**; m.p. 118-120 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3400, and 1380: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.30-1.54 (m, 9H), 1.60 (s, 3H), 1.66-2.86 (m, 9H), 3.10-3.14 (m, 2H), 6.66 (m, 1H), 7.08-7.42 (m, 4H), 7.86 (m, 1H), 9.00 (broad, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 19.22, 26.74, 27.56, 29.82, 31.12, 35.32, 35.34, 36.78, 44.98, 48.94, 110.82, 114.02, 114.70, 118.22, 119.00, 120.96, 123.58, 125.62, 132.30, 134.32, 134.85, 145.12, 148.48, 153.30 ppm. EI-MS m/z: 388.17 Anal. Calcd. for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21; O, 12.36. Found: C, 74.16; H, 6.18.

Method B:

In a round bottom flask (10 ml), indol-estrone (200 mg, 0.51 mmol), anhydride acetic (1ml) and nitric acid (1 ml), were stirring to room temperature for 12 h. crystallization using the methanol:hexane:water (4:2:1) system to give a nitro-steroid-indol derivative (44% yield); ^{1}H NMR and 13C NMR spectra were determined and were compared with method A product..

Preparation of N-((2-hydroxynaphthalen-1-yl)((2-(((8aS)-8a-methyl-5-nitro-1,2,6b,7,8,8a,9,14,14a,14b-decahydronaphtho [2',1':4,5]indeno[1,2-b]indol-4-yl)amino)ethyl)amino)methyl) acetamide (4).

In a round bottom flask (10 ml), compound **3** (0.50 mmol), 2-hydroxy-1-naphthaldehyde (90 mg, 0.52 mmol), ethylenediamine (60 mg, 0.75 mmol) and 4 ml of acetonitrile:ethanol (3:1) were stirred to reflux temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:1) system.

yielding 54 % of **4**, m.p. 76-78 ^{o}C; IR (V_{max}, cm^{-1}) 3432, 3398, 1648 and 1380: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.30-1.54 (m, 2H), 1.60 (s, 3H), 1.66-1.86 (m, 2H), 1.98 (s, 3H), 2.00-2.06 (m, 4H), 2.50-2.56 (m, 2H), 2.86-3.14 (m, 5H), 3.52 (m, 2H), 6.52 (m, 1H), 6.80 (m, 1H), 7.08-7.22 (m, 2H), 7.24 (m, 1H), 7.26 (m, 1H), 7.32 (m, 1H), 7.42 (m, 1H), 7.58-7.80 (m, 4H), 7.88 (broad, 5H), 8.22 (m, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 23.56, 24.40, 26.74, 27.56, 28.94, 31.13, 35.31, 36.42, 36.78, 45.00, 47.10, 47.32, 48.70, 60.68, 111.64, 113.16, 114.60, 117.50, 117.54, 118.60, 119.30, 119.78, 124.15, 124.24, 125.10, 128.60, 126.88, 128.94, 129.34, 131.44, 136.38, 140.02, 140.75, 140.96, 147.36, 151.02, 152.44, 170.12 ppm. EI-MS m/z: 643.31 Anal. Calcd. for $C_{39}H_{41}N_5O_4$: C, 72.76; H, 6.42; N, 10.88; O, 9.94. Found: C, 72.68; H, 6.36.

N-{37-methyl-4-oxa-16,19,35-triazanonacyclo[20.18.0.$0^{3,20}$.$0^{5,14}$.$0^{8,13}$.$0^{25,40}$.$0^{26,37}$.$0^{28,36}$.0^{29}…1(22),2,5(14),6,8(13),9,11,20,28(36), 29(34),30,32-dodecaen-15-yl}acetamide (5)

In a round bottom flask (10 ml), compound **4** (0.50 mmol), potassium carbonate (50 mg, 0.36 mmol) and 4 ml of dimethyl sulfoxide were stirred to reflux temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:hexane:water (4:1:1) system.

yielding 66 % of **5**, m.p. 60-62 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 1650 and 1112: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.30-1.54 (m, 2H), 1.60 (s, 3H), 1.66-1.86 (m, 2H), 1.96 (s, 3H), 2.00-2.86 (m, 6H), 3.06 (m, 2H), 3.08-3.12 (m, 3H), 3.60 (m, 2H), 4.40 (m, 1H), 6.30-6.36 (m, 2H), 6.96 (m, 1H), 7.00 (broad, 4H), 7.08-7.26 (m, 3H), 7.34 (m, 1H), 7.44 (m, 1H), 7.66-8.06 (m, 4H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C:23.56, 24.40, 26.74, 27.56, 28.94, 31.13, 35.31, 36.42, 36.78, 44.90, 45.40, 47.32, 48.18, 70.14, 107.63, 111.64, 117.51, 118.60, 119.00, 119.28, 119.78, 121.44, 123.36, 123.73, 124.35, 125.10, 125.78, 128.18, 129.12, 129.22, 129.38, 129.88, 130.14, 137.68, 140.72, 140.75, 147.26, 152.42, 170.12 ppm. EI-MS m/z: 596.31 Anal. Calcd. for $C_{39}H_{40}N_4O_2$: C, 78.49; H, 6.76; N, 9.39; O, 5.36. Found: C, 78.00; H, 6.70.

6-(N-{37-methyl-4-oxa-16,19,35-triazanonacyclo[20.18.0.$0^{3,20}$.$0^{5,14}$.$0^{8,13}$.$0^{25,40}$.$0^{26,37}$.$0^{28,36}$.0^{29}…1(22),2,5(14),6,8(13),9,11,20, 28(36),29(34),30,32-dodecaen-15-yl}acetamido)hex-5-ynoic acid (6)

In a round bottom flask (10 ml), compound **5** (0.50 mmol), 5-hexynoic acid (61 µl, 0.54 mmol), Copper(II) chloride anhydrous (70 mg, 0.52 mmol) in 5 ml of methanol were stirred to room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:bencene:water (4:1:1) system.

yielding 45 % of **6**, m.p. 128.130 ^{o}C; IR (V_{max}, cm^{-1}) 3432, 1702, 1650 and 1112: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.30-1.54 (s, 3H), 1.58 (s, 3H), 1.66-1.86 (m, 2H), 1.88 (m, 2H), 2.00-2.06 (m, 4H), 2.22 (s, 3H), 2.32 (m, 2H), 2.47 (m, 2H), 2.48 (m,4H), 2.60-3.12 (m, 5H), 3.22-3.70 (m, 4H), 4.40 (m, 1H), 6.32-6.38 (m, 2H), 6.90 (broad, 4H), 694 (m, 1H), 7.08-7.25 (m, 3H), 7.34 (m, 1H), 7.44 (m, 1H), 7.64-8.06 (m, 4H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.82, 20.86, 22.04, 24.38, 26.74, 27.56, 28.94, 31.13, 32.60, 35.30, 36.42, 36.78, 43.16, 45.42, 47.32, 47.93, 61.00, 76.74, 87.41, 107.60, 111.64, 117.51, 118.60, 119.00, 119.28, 119.78, 121.22, 123.14, 123.74, 124.32, 125.13, 125.82, 127.13, 129.14, 129.34, 129.60, 130.16, 130.35, 137.68, 140.72, 140.78, 149.42, 152.42, 170.10, 178.40 ppm. EI-MS m/z: 706.35 Anal. Calcd. for $C_{45}H_{46}N_4O_4$: C, 76.46; H, 6.56; N, 7.93; O, 9.05. Found: C, 76.40; H, 6.50.

2methyl-1-{37-methyl-4-oxa-16,19,35-triazanonacyclo[20.18.0.$0^{3,20}$.$0^{5,14}$.$0^{8,13}$.$0^{25,40}$.$0^{26,37}$.$0^{28,36}$.0^{29}…1(22),2,5(14),6,8(13),9,11,20, 28(36),29(34),30-32-dodecaen-15-yl}1,3,6-triazacyclododec-2-en-11-yn-7-one (7)

In a round bottom flask (10 ml), compound **6** (0.5 mmol), ethylenediamine (60 mg, 0.75 mmol), boric acid (40 mg, 0.61 mmol) and 4 ml of methanol were stirred to room temperature for 48 h. The solvent of the mixture obtained was removed under reduced pressure and purified through a crystallization using the methanol:water (4:1) system.

yielding 56 % of **7**, m.p. 167-169; IR (V_{max}, cm^{-1}) 3432, 3330, 1650 and 1114: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.30-1.54 (s, 3H), 1.57 (m, 1H), 1.58 (m, 2H), 1.60 (s, 3H), 1.66-1.86 (m, 2H), 1.90 (m, 2H), 1.98 (s, 3H), 2.00-2.06 (m, 4H), 2.08 (m, 2H), 2.60-2.86 (m, 2H), 2.90 (m, 2H), 2.94 (m,1H), 3.08-3.12 (m, 3H), 3.22 (m, 2H), 3.58-3.92 (m, 4H), 5.56 (broad, 3H), 6.30-6.34 (m, 2H), 6.88 (m, 1H), 7.08-7.25 (m, 3H), 7.34 (m, 1H), 7.40 (broad, 1H), 7.44 (m, 1H), 7.82-8.04 (m, 4H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 19.12, 19.90, 24.38, 25.14, 26.77, 27.56, 28.94, 31.13, 35.34, 35.75, 36.42, 36.78, 38.90, 45.20, 45.42, 47.32, 49.50, 57.17, 66.92, 75.78, 81.52, 107.60, 111.60, 117.51, 118.62, 119.00, 119.28, 119.78, 122.00, 123.92, 124.35, 124.50, 125.10, 126.54, 127.55, 128.00, 128.62, 129.40, 129.88, 130.14, 137.68, 140.72, 141.46, 148.22, 150.90, 152.42, 167.60 ppm. EI-MS m/z: 730.39 Anal. Calcd. for $C_{47}H_{50}N_6O_2$: C, 77.23; H, 6.89; N, 11.50; O, 4.38. Found: C, 77.18; H, 6.80.

Electronic parameters evaluation (HOMO and LUMO).

The molecular orbitals HOMO and LUMO for all compounds were theoretically evaluated with SPARTAN'06 software package (Wavefunc-tion Inc. Irvine, CA, 2000), using Hartree-fock method at 321-G level [17].

Theoretical evaluation of the interaction between compounds 3 or 7 with aromatase.

Theoretical analysis of interaction of compounds 2-7 on aromatase protein (4kq8) was carried out using a docking program (DockingServer) [18]. In addition, anastrazol, letrozole, exametane were used as controls.

3. RESULTS SECTION

Several compounds have prepared as aromatase inhibitors [10-14]; nevertheless, their interaction with enzyme surface is very confusing; therefore, several studies are needed to evaluate this phenomenon. The objective of this study was to synthesize and evaluate their interaction with the aromatase enzyme using a docking model. [18].

First stage

Synthesis of a steroid-indeno-indol-4-ol-acetamide derivative

There are some studies which showed the preparation of several indole analogs using some reagents such as rhodium [19], palladium [20], phosphine [21], Cu(II) [22], Cobalt(III) [23] and others; However, the handling of some of these reagents requires special conditions and they are also very expensive. In this study, a steroid-indeno-indol-4-ol (**3**) derivative was prepared (Figure 1) by the reaction of 2-nitroestrone with phenylhydrazine in acid medium (Method A) or via nitration of compound **2** (steroid-indole derivative) with nitric acid/anhydride acetic to form **3**. It is noteworthy that Method A showed a higher yield compared with Method B. ^{1}H NMR spectra for **3** shown some bands at 0.64 ppm for methyl group which bound to steroid nucleus; at 1.60 ppm for methyl group; at 1.30-1.54, 1.66-6.66 and 7.85 ppm for steroid nucleus; at 7.09-7.43 ppm for indol ring; at 8.96 for hydroxyl group. ^{13}C NMR spectrum for **3** showed some signals at 19.26 ppm for methyl group; at 26.77-48.98, 114.05, 123.56, 132.33-134.33 and 145.09-148.48 ppm for steroid nucleus; at 110.84, 114.72-120.96, 125.60, 134.85 and 153.34 ppm for indol ring. Finally, the mass spectrum from **3** showed a molecular ion (m/z) at 388.17.

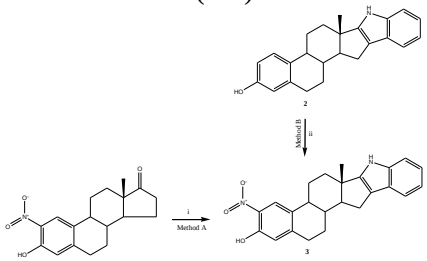

Figure 1. Preparation of a 5-nitro-indol-steroid-acetamide derivative (**3**). Reaction of 2-nitroestrone (**1**) with phenylhydrazine (Method A) to form **3**; also **3** was prepared (Method B) from an indol-estrone derivative (**2**). i = acetic acid; ii = nitric acid/anhydride acetic; iii = ethanol/rt

Synthesis of naphthalen-nitro-steroid-indol-acetamide complex (4)

There are some studies which indicate the preparation of several acetamide analogs using some reagents such as triazole derived [24], proline [25], 4-(4-morpholinyl)benzenamine [26], hydroxy-benzotriazole [27]. However, in this investigation the compound **4** was prepared (Figure 2 and 3) using the multi-component system (compound **3**, acetonitrile, 2-Hydroxy-naphthalene-1-carbaldehyde and ethylene- diamine), it should be noted that no special reagent was required for the preparation of **4**. The results of ^{1}H NMR spectrum of **4** shown some bands at 1.30-1.54, 1.66-1.86, 2.00-2.06, 2.85-3.14, 6.52 and 8.22 ppm for steroid moiety; at 1.60 ppm for methyl group bound to steroid nucleus; at 1.98 ppm for methyl bound to amide group; at 2.04, 2.50-2.57 and 3.54 ppm for methylene groups bound to both amino groups; at 6.80 ppm for amide group; at 7.08-7.22, 7.28 and 7.42 ppm for indol ring; at 7.24, 7.32 and 7.58-7.80 ppm for naphthalene group; at 7.88 ppm for both hydroxyl and amino groups. The ^{13}C NMR spectra showed chemical shifts at 23.56 ppm for methyl group bound to amide; at 24.38 ppm for methyl group bound to steroid nucleus; at 26.74-45.00, 47.32, 114.60, 124.24, 131.44-140.02 and 147.36 ppm to steroid moiety; at 47.10-48.70 ppm for methylene bound to both amino groups; at 60.68 ppm for methylene group bound to both amide and amino groups; at 111.64, 117.50, 118.60-119.78, 125.10, 140.75 and 152.44 ppm for indole ring; at 113.16, 117.54, 124.15, 126.88-129.34, 140.96 and 151.02 ppm for naphthalene group; at 170.12 ppm for amide group. In addition, **4** showed a molecular ion (m/z) at 643.31.

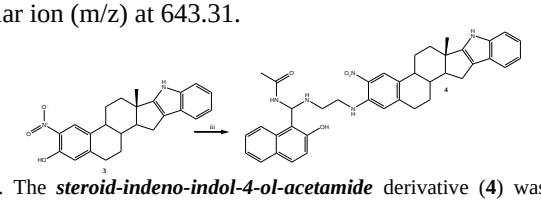

Figure 2. The ***steroid-indeno-indol-4-ol-acetamide*** derivative (**4**) was prepared using the multicomponent system (compound **3**, 2-hydroxy-1-naphthaldehyde, ethylenediamine, acetonitrile). iii = acetonitrile:ethanol.

Preparation of a triazanonacyclo-dodecaen-acetamide derivative via etherification (5)

Several ether derivatives have been synthesized through displacement of nitro groups using some reagents such as methoxy groups [28], fluoride ion [29], nitropropane or nitrocyclohexanone [30], sodium phenoxide [31], nitrobenzamide in DMSO [32]. Therefore, the formation of ether group (compound **5**) was carried out by an internal reaction with dimethyl sulfoxide under mild conditions (Figure 3) using previously reports for preparation of ether groups [33].

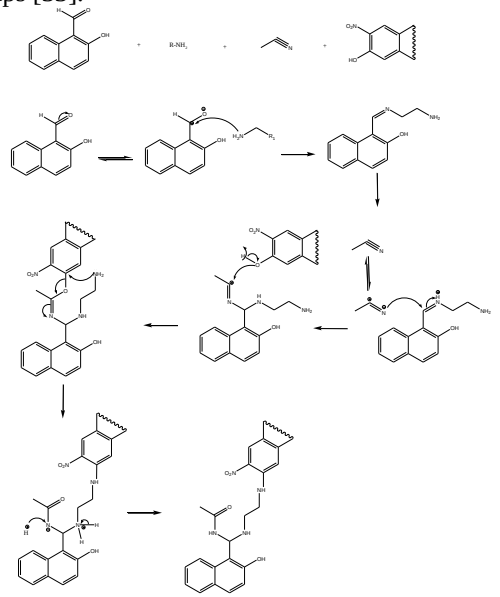

Figure 3. Reaction mechanism for the formation of 5-nitro-indol-steroid-acetamide derivative (compound **4**).

^{1}H NMR spectra for **5** showed several signals at 1.30-1.54, 1.66-1.86, 2.00-2.86, 3.08-3.12 and 6.30-6.36 ppm for steroid moiety; at 1.60 ppm for methyl group bound to steroid nucleus; at 1.96 ppm for methyl bound to amide group; at 3.06, 3.60 and 4.40 ppm for methylene groups bound to both amino groups; at 7.00 ppm for amino and amide groups; at 7.08-7.26 and 7.44 ppm for indole ring; at 6.96, 7.34 and 7.66-8.06 ppm for naphthalene group. ^{13}C NMR spectrum for **5** showed several signals at 23.56 ppm for methyl group bound to amide; at 24.40 ppm for methyl group bound to steroid nucleus; at 26.74-36.78, 45.40-47.32, 107.63, 119.00, 129.38 and 130.14-140.72 ppm for steroid moiety; at 44.90 and 48.18-70.14 ppm for methylene groups bound to both amide and amino groups; at 111.64-118.60, 119.28-119.78, 125.19, 140.75 and 152.42 ppm for indol ring; at 121.44, 124.35, 125.78-129.22, 129.88 and 147.26 ppm for naphthalene group; at 170.12 ppm for amide group. Finally, the mass spectrum from **5** showed a molecular ion (m/z) at 596.31.

Addition of an amide derivative (5) to alkyne group to form 6.

There are some reports on addition of amide to alkyne groups using several reagents such as platinum [34], ruthenium [35], nickel [36], Rhodium/Copper [37] palladium(II) [38] and others. In this study, a triazanonacyclo-acetamido-hex-5-ynoic acid derivative (**6**) was prepared (Figure 3) via reaction of **5** with 5-hexynoic acid in presence of Copper(II). ^{1}H NMR spectra for **6** showed several signals at .130-1.54, 1.66-1.86, 2.00-2.06, 2.60-3.12 and 6.32-6.38 ppm for steroid moiety; at 1.58 ppm for methyl bound to steroid nucleus; at 2.22 for methyl bound to amide group; at 1.88 and 2.32-2.47 ppm for methylene groups bound to both carboxyl and alkyne groups; at 3.22-4.40 ppm for methylene groups bound to both amino and amide groups; at 6.90 ppm for both amino and carboxyl groups; at 6.94, 7.34 and 7.64-8.06 for naphthalene ring; at 7.08-7.25 and 7.44 ppm for indole ring. ^{13}C NMR spectra for **6** showed several signals at 15.82-20.86 and 32.60 ppm for methylene groups bound to both alkyne and carboxyl groups; at 24.38 ppm for methyl group bound to amide group; at 22.04 ppm for methyl group bound to steroid nucleus; at 26.74-31.13, 35.30-36-78, 45.42-47.32, 107.60, 119.00, 129.34, 130.16 and 137.68-140.72 ppm for steroid moiety; at 43.26, 47.93 and 76.74 ppm for methylene groups bound to both amide and amino groups; at 61.00 and 87.41 ppm for alkyne group; at 111.64-118.60, 119.28-119.78, 125.13, 140.78 and 150.42 ppm for indole ring; at 121.22-124.32, 125.82-129.14, 129.60 and 130.35-149.42 ppm for naphthalene group; at 170.10 ppm for amide group; at 178.40 ppm for carboxyl group. In addition, **6** showed a molecular ion (m/z) at 706.35.

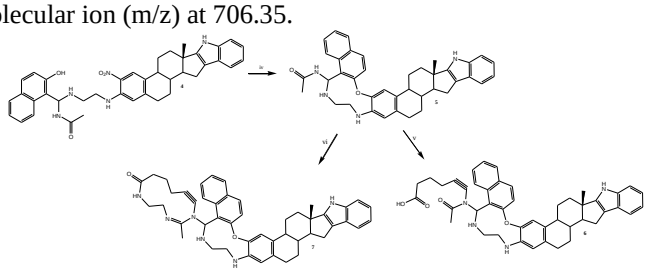

Figure 3. Steroid-triazanonacyclo-7-one (7). Reaction of a (5-nitro-indol-steroid-acetamide derivative (4) with dimethylsulfoxide (iv) to form a steroid-triazanonacyclo-acetamide (5). Then 5 was reacted with 5-hexynoic acid (v) to formation of the steroid-triazanonacyclo-dodecaen-15-yl}acetamido)hex-5-ynoic acid complex (6). Finally, 6 was reacted with ethylenediamine in presence boric acid (vi).

Preparation of a triazacyclododec-2-en-11-yn-7-one derivative

Several triazacyclododecen analogs have been synthesized using some reagents such as Copper(II) [39], Nickel [40], Iron(III) [41], *n*-butyllithium [42]. In this investigation, **6** reacted with ethylenediamine using boric acid as catalyst (Figure 3) to form the triazacyclododec-2-en-11-yn-7-one (**7**). It is important to mention that the use of this reagent does not require special conditions. [43]. ^{1}H NMR spectra for **7** display some signals at 1.30-1.54, 1.66-1.86, 2.00-2.06, 2.60-2.86, 3.08-3.12 and 6.30-6.34 ppm for steroid moiety; at 1.60 ppm for methyl bound to steroid nucleus; at 1.98 ppm for methyl bound to amide group; at 1.58, 1.90, 2.08 and 3.58-3.92 ppm for methylene groups involved in 1,3,6-triaza-cyclododec-2-en-11-yn-7-one system; at 2.90-2.94 and 3.22 ppm for methylene groups bound to both amino groups; at 5.56 ppm for amino groups; at 7.08-7.25 and 7.44 ppm for indol ring; at 6.88, 7.34 and 7.82-8.04 ppm for naphthalene ring. ^{13}C NMR spectrum showed several signals for **7** at 19.12, 25.14, 35.75, 38.90, 45.42-47.32 and 57.17 and 75.788 ppm for methylene groups involved in 1,3,6-triaza-cyclododec-2-en-11-yn-7-one system; at 19.90 ppm for methyl bound to imino group; at 24.38 ppm for methyl bound to steroid nucleus; at 26.77-25.34, 36.42-36.78, 107.60, 119.00, 129.40, 130.14-137.68 and 141.46 ppm for steroid moiety; at 45.20-49.50 and 75.78 ppm for methylene groups bound to both amino groups; at 69.92 and 81.52 ppm for alkyne group; at 111.60-118.62, 119.28-119.78, 125.10, 140.72 and 152.42 ppm for indol ring; at 122.00-124.50, 126.54-128.62 and 148.22 ppm for naphthalene ring; at 150.90 ppm for imino group; at 167.60 ppm for amide group. Additionally, the mass spectrum from **7** display a molecular ion (m/z) at 730.39.

Second stage

Physicochemical parameters of compounds 3-7.

It is noteworthy that some physicochemical factors, such as logP and π have be used to evaluate the degree of lipophilicity of a molecule [44, 45]. It is important to mention, these parameters were determined for compounds **2-7**. The results (Table 1 and 2) indicate that logKow and π were higher for compound **7** compared to **2-6**, which translates to more lipophilicity degree (Table 1). However, it is noteworthy that this phenomenon could be conditioned by other parameters chemical such as molar volume (V_m) and refractivity molar (R_m) which have been relationship with biological activity of some drugs [48]; these physicochemical factors are tools which can used to identify different chemical characteristics that depend of substituents of a specific molecule.To evaluate both V_m and R_m descriptors for compounds **2-7** a previously method reported was used [49]; the results showed that V_m and R_m were higher for both **6** and **7** compared with the compounds **2-5** (Table 3). These data suggest that the steric hindrance and the different conformations involved in compounds **6** or **7** could be determining factors in the biological activity exerted by these steroid derivatives in some biological model.

Table 1. Physicochemical parameters involved in the chemical structure of compounds **2-4**.

2	$-CH_3$ [aliphatic carbon]	0.5473
	$-CH_2-$ [aliphatic carbon]	2.4555
	-CH [aliphatic carbon]	1.0842
	Aromatic Carbon	4.1160
	-OH [hydroxy, aromatic attach]	**-0.4802**
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.2676
	Fused aliphatic ring unit correction	-1.3684
	Equation Constant	0.2290
	π	2.8948
	Log Kow	6.3248
3	$-CH_3$ [aliphatic carbon]	0.5473
	-CH2- [aliphatic carbon]	2.4555
	-CH [aliphatic carbon]	1.0842
	Aromatic Carbon	4.1160
	-OH [hydroxy, aromatic attach]	-0.4802
	$-NO_2$ [nitro, aromatic attach]	-0.1823
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.2676
	Ring reaction -> -NO2 with -OH/amino/azo	0.5777
	Fused aliphatic ring unit correction	-1.3684
	Equation Constant	0.2290
	π	0.3954
	Log Kow	6.7202
4	$-CH_3$ [aliphatic carbon]	1.0946
	$-CH_2-$ [aliphatic carbon]	3.4377
	-CH [aliphatic carbon]	1.4456
	-NH- [aliphatic attach]	-2.9924
	Aromatic Carbon	7.0560
	-OH [hydroxy, aromatic attach]	-0.4802
	-N [aliphatic N, one aromatic attach]	-0.9170
	$-NO_2$ [nitro, aromatic attach]	-0.1823
	-C(=O)N [aliphatic attach]	-0.5236
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.2676
	Ring reaction -> -NO2 with -OH/amino/azo	0.5777
	Fused aliphatic ring unit correction	-1.3684
	Equation Constant	0.2290
	π	0.3979
	LogKow	7.1181

Electronic parameters evaluation of both highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

There are some reports which suggest that both HOMO) and LUMO are two factors involved in biological activity of some drugs [48]. Therefore, in this investigation both HOMO and LUMO were evaluated (Table 3) using Spartan software [49]. The results showed in table 3 indicated that HOMO values were higher for **6** compared with the compounds **2-5** and **7**; these data indicate that **6** exert strong electro donating ability compared with **2-5** and **7**. In addition, these results suggest that **6** could induce changes in some biological system compared to **2-5** in a similar way with other types of molecules [48].

It is noteworthy that there are some studies suggest that other physicochemical factors are involved in the activity of several drugs, such as hydrogen bond donor groups. (HBD) and hydrogen bond acceptor groups (HBA) which may exert also changes on some biological system [50]. In this regard, these physicochemical descriptors have been evaluated using some pharmacophore models [51, 52];

Table 2. Physicochemical factors from compounds **5-7**.

5	$-CH_3$ [aliphatic carbon]	1.0946
	$-CH_2-$ [aliphatic carbon]	3.4377
	-CH [aliphatic carbon]	1.4456
	-NH- [aliphatic attach]	-2.9924
	Aromatic Carbon	7.0560
	-N [aliphatic N, one aromatic attach]	-0.9170
	-O- [aliphatic O, two aromatic attach]	0.2923
	-C(=O)N [aliphatic attach]	-0.5236
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.2676
	Fused aliphatic ring unit correction	-1.3684
	Equation Constant	0.2290
	π	
	Log Kow	7.4952
6	$-CH_3$ [aliphatic carbon]	1.0946
	$-CH_2-$ [aliphatic carbon]	4.9110
	-CH [aliphatic carbon]	1.4456
	#C [acetylenic carbon]	0.2668
	-NH- [aliphatic attach]	-1.4962
	-N< [aliphatic attach]	-1.8323
	Aromatic Carbon	7.0560
	-N [aliphatic N, one aromatic attach]	-0.9170
	-O- [aliphatic O, two aromatic attach]	0.2923
	-COOH [acid, aliphatic attach]	-0.6895
	-C(=O)N [aliphatic attach]	-0.5236
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.2676
	Fused aliphatic ring unit correction	-1.3684
	Equation Constant	0.2290
	π	0.3771
	Log Kow	8.2097
7	$-CH_3$ [aliphatic carbon]	1.0946
	$-CH_2-$ [aliphatic carbon]	5.8932
	-CH [aliphatic carbon]	*1*.4456
	C [aliphatic carbon - No H, not tert]	0.9723
	#C [acetylenic carbon]	0.2668
	-NH- [aliphatic attach]	-2.9924
	-N< [aliphatic attach]	-1.8323
	Aromatic Carbon	7.0560
	-N [aliphatic N, one aromatic attach]	-0.9170
	-O- [aliphatic O, two aromatic attach]	0.2923
	-C(=O)N [aliphatic attach]	-0.5236
	Aromatic Nitrogen [5-member ring]	-0.5262
	-tert Carbon [3 or more carbon attach]	0.2676
	-N=C [aliphatic attach]	-0.0010
	Fused aliphatic ring unit correction	-1.3684
	-C-N=C-N-C- [cyclic] structure correction	-0.6000
	Equation Constant	0.2290
	π	0.5468
	LogKow	8.7565

Here, it is important to mention some studies suggest that both HBD and HBA can condition some pharmacokinetic process of drugs in the human body []; analyzing this hypothesis, the theoretical data found in is study suggest that compounds **2** to **7** could have the ability of penetrate some barrier biological of human body. However, it is noteworthy that the rule does not predict if a compound could be pharmacologically active; therefore, other type of studies must be carried out to determine the interaction between some compounds with several biological targets such as proteins or enzymes.

It is important to mention that pharmacophores are generally used to evaluate some chemical characteristics that are related with the biological activity of several molecules. Analyzing these data, in this investigation a theoretical study was carried out using a pharmacophore model [53]. The theoretical results (Figure 4-6) showed several hydrogen bond donor groups; such as -OH for the compound **2**; -NH- for **3-7**. Other theoretical data showed several hydrogen bond acceptor groups such as $-NO_2$ for **2**; -OH for both **3** and **4**; -NHCO- for **5** and **6**; =N- for **7**. In addition, other theoretical results (table 3) showed both HBA (< 10) and for HBD (< 5) values for compounds **2** to **7**.

Table 3. Physicochemical parameters of compounds **2-7**.

	Compounds					
Parameter	**2**	**3**	**4**	**5**	**6**	**7**
V_m (cm^3)	277.80	289.70	485.50	448.60	520.60	543.60
R_m (cm^3)	105.56	112.10	189.79	179.20	206.88	215.30
Polarizability	69.08	61.237	68.91	86.41	95.10	53.824
Dipole moment (debyte)	7.73	8.97	9.74	4.32	7.59	10.47
PSA (Å^2)	62.818	61.237	91.391	50.091	64.544	53.834
Energy (au)	1247.82	1247.77	2058.06	1855.10	2333.02	2269.74
HOMO (eV)	-5.97	-6.19	-4.46	-4.50	-3.32	-4.75
LUMO (eV)	1.10	0.95	0.69	1.14	0.64	1.30
Gap energy, eV (HOMO-LUMO)	-7.07	-7.14	-5.15	-5.64	-3.96	-6.05
HBD	2	2	4	2	3	3
HBA	5	5	9	6	8	8

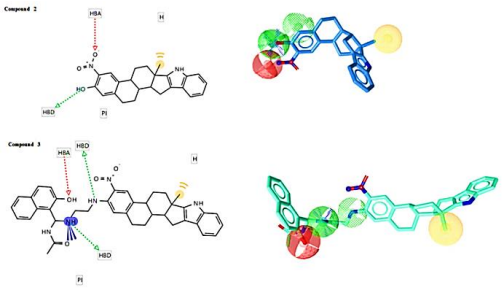

Figure 4. Scheme represents a pharmacophore model from both compounds **2** and **3** using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

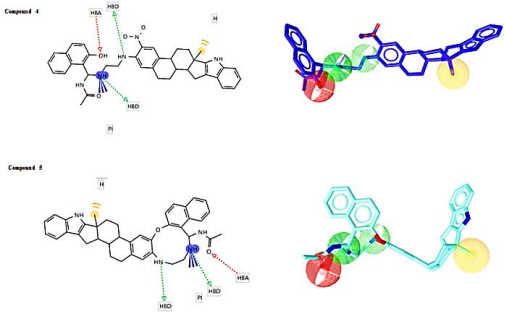

Figure 5. Pharmacophore from both compounds **4** and **5** using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

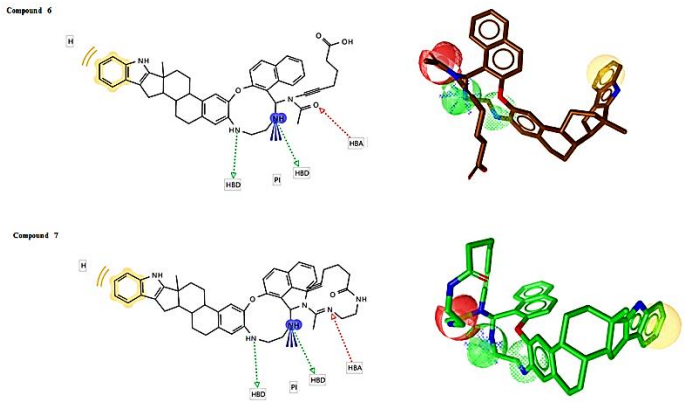

Figure 6. Scheme represents a pharmacophore from both compounds **6** and **7** using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

Evaluation of interaction of compounds 3-7 with aromatase protein (4kq8).

There are some studies that indicate that several substances can interact with some macromolecules which can be translated as the physiological regulation of some enzymes [54]; it is noteworthy that several drugs can exert changes biological activity of specific enzyme. In order, to evaluate this phenomenon some theoretical models have been used to predict the interaction of some drugs with enzymes [55]. Therefore, in this investigation was carried out a theoretical analysis of interaction of compounds **3-7** with aromatase protein (4kq8) [56] using a Docking model [57]. The results shown in figures 7-9 and table 4 indicate the interaction of compounds **2**-7 several with amino acid residues involved in enzyme surface (4kq8).

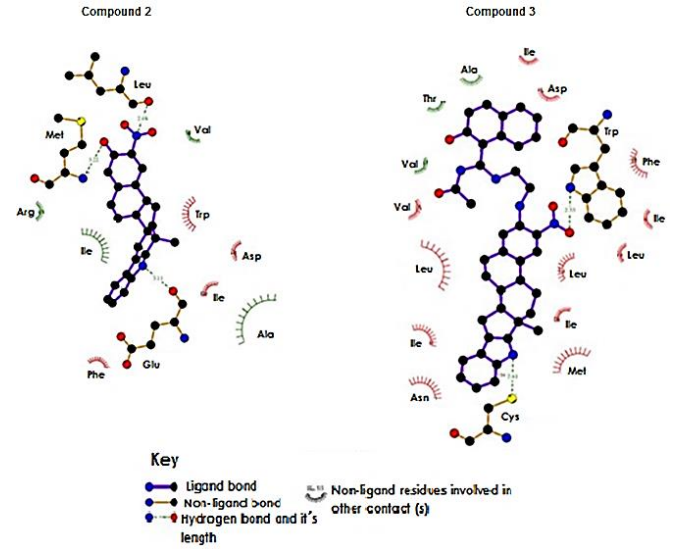

Figure 7. The scheme shows the binding of compounds **2** and **3** with some aminoacid residues of the aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

However, to determine whether compounds **2-7** could act as aromatase inhibitors; also, theoretical interaction of enzyme with some aromatase antagonists, such as anastrozole, letrozole and exemestane was evaluated. The results (Figures 7-9 and Table 4) showed that anastrozole could interact with several aminoacid residues such as Ile_{133}, Phe_{134}, Phe_{221}, Ala_{306}, Asp_{309}, Thr_{310}, Val_{310}, Val_{373}, Met_{374}, Leu_{477} and Ser_{478} which are involved in the aromatase (4kq8 protein) surface. It is noteworthy that also **6** could bind to these types of aminoacid residues; however, only some of these aminoacid residues may participate in the interaction between 4kq8 protein with compounds **2-5** and **7**; this phenomenon could involve other type intramolecular interactions due to changes in the energy levels.

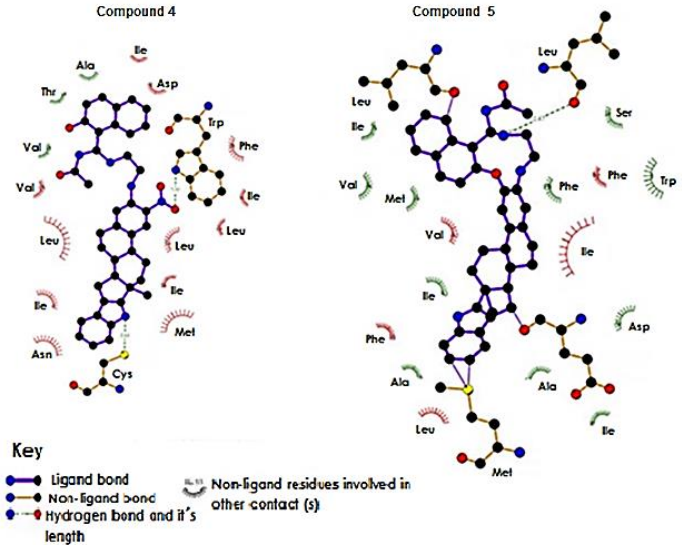

Figure 8. The scheme shows the binding sites of compounds **4** and **5** with some amino acid residues of aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

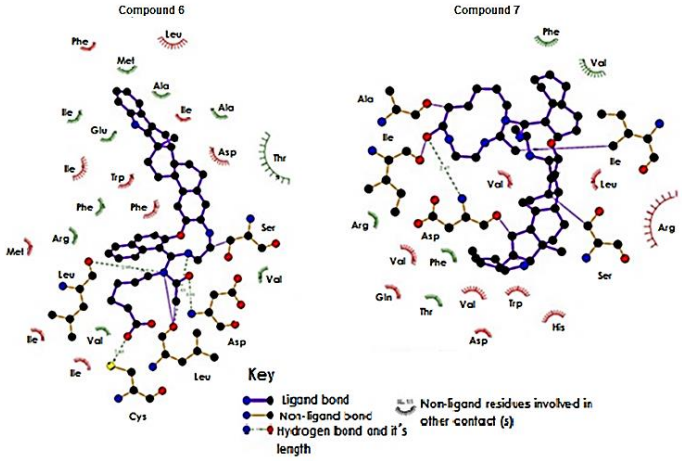

Figure 9. The scheme shows the binding of compounds **6** and **7** with some aminoacid residues of the aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

Thermodynamic parameters

There are some reports which indicate that several thermodynamic factors may be involved in the interaction drug-protein [41]; therefore, a theoretical ass was carried out on some thermodynamic parameters involved in the interaction of anastrazol, letrozole, exametane and the compounds **2-7** with the 4kq8 protein such as 1) free energy of binding which determinate the energy value that require a molecule to interact with a protein in a water environment. 2) Electrostatic energy that is the product of electrical charge and electrostatic potential, which are involved in the ligand-protein system [58]; 3) total intermolecular energy and 4) Van der Waals (vdW) + hydrogen bond (Hbond) + desolvation energy (Desolv. Energy; which have an influence on the movement of water molecules into or out of the ligand-protein system) [58] using a theoretical model (dockingserver) [57].

Table 4. Residues aminoacids involved in the interaction between anastrazol, letrozole, exametane and compounds **2-7** with 4kq8 protein.

Arg_{115} Ile_{133} Phe_{134} Phe_{221} Ala_{306} Asp_{309} Thr_{310} Val_{310} Val_{373} Met_{374} Leu_{477} Ser_{478}	Arg_{115} Ile_{133} Phe_{134} Phe_{221} Trp_{224} Ala_{306} Asp_{309} Thr_{310} Val_{370} Leu_{477}	Arg_{115} Ile_{133} Phe_{134} Phe_{221} Trp_{224} Ala_{306} Asp_{309} Thr_{310} Val_{369} Val_{373} Ser_{478}	Arg_{115} Ile_{133} Phe_{148} Trp_{224} Glu_{302} Ile_{305} Ala_{306} Asp_{309} Val_{370} Leu_{372} Met_{374}	Ile_{70} Cys_{74} Ile_{133} Phe_{134} Trp_{224} Leu_{228} Ile_{305} Ala_{306} Asp_{309} Thr_{310} Val_{370} Leu_{372} Val_{373} Met_{374} Ile_{395} Asn_{397} Leu_{477}	Ile_{70} Arg_{115} Ile_{133} Phe_{134} Phe_{148} Phe_{221} Trp_{224} Leu_{228} Asp_{309} Thr_{310} Val_{369} Val_{370} Leu_{372} Val_{373} Met_{374} Leu_{477} Ser_{478} His_{480}	Ile_{132} Ile_{133} Phe_{134} Phe_{148} Leu_{152} Phe_{221} Trp_{224} Glu_{302} Met_{303} Ile_{305} Ala_{306} Asp_{309} Val_{370} Leu_{372} Val_{373} Met_{374} Ile_{398} Ala_{438} Leu_{477} Ser_{478}	Cys_{74} Arg_{115} Ile_{132} Ile_{133} Phe_{134} Phe_{148} Leu_{152} Phe_{221} Trp_{224} Glu_{302} Met_{303} Ile_{305} Ala_{306} Asp_{309} Thr_{310} Val_{370} Asp_{371} Leu_{372} Val_{373} Met_{374} Ile_{395} Ala_{438} Leu_{477} Ser_{478}	Ile_{70} Cys_{74} Met_{127} Ile_{133} Phe_{134} Phe_{148} Trp_{224} Glu_{302} Met_{303} Ile_{305} Ala_{306} Thr_{310} Asp_{371} Leu_{372} Met_{374} Ile_{395} Asn_{397} Cys_{437} Leu_{477}

*Similar residues aminoacids (red); Different residues aminoacids (blue and green).

Table 5. Thermodynamic factors involved in the interaction of anastrazol, letrozole, exametane and compounds **2-7** on aromatase (4kq8).

Compound	Est. Free Energy of Binding (kcal/mol)	vdW + Hbond + desolv. Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Intermol. Energy (kcal/mol)	Interact. Surface
Antrazol	-9.58	-11.41	0.01	-11.41	569.117
Letrozan	-8.67	-9.80	-0.10	-9.90	556.04
Exemestan	-10.61	-10.73	-0.08	-10.81	511.069
2	17.88	17.88	-0.30	17.59	613.343
3	302.49	282.99	-0.25	282.74	907.966
4	296.90	259.15	-0.09	259.07	872.307
5	455.63	455.74	-0.41	455.33	901.322
6	699.38	685.02	-0.32	684.70	996.294
7	926.66	927.57	-1.35	926.22	1018.609

The results showed in the table 5 indicate that all thermodynamic parameters were higher for compound **6** compared with anastrazol, letrozole, exametane and compounds **2-5**; however, these parameters were low in comparison with **7**. This phenomenon indicates that there are differences in the energy levels between the interaction of the compounds studied and the 4kq8 protein, which can be translated as changes in the biological activity of aromatase in the presence of **6** in comparison with the compounds **2-5** and **7**.

4. CONCLUSIONS

Theoretical data indicate that compound **6** could be a good candidate as aromatase inhibitor which translates as a possible drug for breast cancer. Nevrtheless, it is noteworthy that it is necessary to evaluate their activity in some biological model.

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Preparation of two steroid derivatives and its theoretical interaction with a 17β-hydroxysteroid dehydrogenase type 1

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ABSTRACT

The aim of this study was synthesizing two steroid derivatives to evaluate their theoretical interact with a 17β-hydroxysteroid dehydrogenase type 1. The first stage was achieved by the preparation of a steroid-imino analog (compound **2**) using a reaction of imination and *ii*) etherification. Then, the theoretical interact of two steroid analogs with 17β-hydroxysteroid dehydrogenase type 1 (1IOL) was evaluated using fisetin and methyl paraben as controls in a docking model. The results suggest that steroid derivatives could interact via a different type of aminoacid residues of 1IOL protein surface. However, the compound **2** showed a constant of inhibition lower compared with fisetin, methyl paraben and compound **3**. All these data indicate that steroid derivative could act as 17β-hydroxysteroid dehydrogenase type 1 inhibitor.

Keywords: *17β-hydroxysteroid hydrogenase, fisetin, paraben, docking.*

1. INTRODUCTION

Cancer has increased in recent years around the world in both women and men; this clinical pathology could be conditioned through of changes in the biological activity of some enzymes such as 17β-hydroxysteroid dehydrogenase (17β-HD) [1-3]. For example, a study showed that increasing levels of estradiol can induce the activation of 17β-HD type 1, which results in a higher risk of breast cancer [4]. Other data indicate that 17β-HD type 7 can induce changes in binding immunoglobulin protein (GRP-78) levels in breast cancer cells (MCF-7) [5]. In addition, a report shown that 17β-HD type 5 modulates the biological activity of both inhibitor binding immunoglobulin and tumor-secreted protein phosphoglycerate kinase 1 on MCF-7 cells [6]. Also, there are some studies which display that 17β-HD Type 2 is correlated with androgen-receptor activation in HEC-1A cells [7]. Other study showed that 17β-hydroxysteroid dehydrogenase Type 1 can stimulate breast cancer through dihydrotestosterone inactivation [8]. Here it is important to mention that in the search of some pharmacological therapy, several 17β-HD type 1 inhibitors have been developed; for example, the preparation of 3-(2-bromoethyl)-16β-(m-carbamoylbenzyl)-17β-hydroxy-1,3,5(10)-estratriene as 17β-HD type 1 inhibitor for breast cancer and endometriosis [9]. Other data showed the Pd-Catalyzed microwave-assisted synthesis of phosphonated 13α-estrones as a potential 17β-HSD type 1 inhibitor [10]. In addition, a study showed the addition of chlorine, bromine or iodine onto positions 2 and/or 4 of 17-deoxy-13α-estrone as potent 17β-HSD type 1 inhibitors using human placenta [11]. Other study showed the preparation of (3α,5α,17α)-3-Hydroxy-3-{[4-(3-methoxybenzyl)piperazin-1-yl]methyl}-andros-Androstan-17-ol from dihydrotestosterone as 17β-HSD type 10 inhibitor using an *in vitro* model [12]. All these data suggest that some drugs can be used as 17β-HSD type 1 inhibitors; however, the interaction of some drugs with an enzyme is very confusing, perhaps this phenomenon could be due to; 1) differences in the chemical structure of each drug; or 2) to different methods used in each experiment. Therefore, the aim of this study was to synthesize two steroid derivatives to evaluate its theoretical interaction with 17β-HSD type 1 using a docking model.

2. MATERIALS AND METHODS

General methods.

The estradiol-ethylenediamine derivative was prepared using a previous method reported [13]. In addition, all the reagents used in this study were purchased from Sigma-Aldrich Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were determined using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{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.

Chemical Synthesis.

Preparation of 7-hydroxy-6-{[(2-{[(2-hydroxynaphthalen-1-yl)methylidene]amino}ethyl) amino]methyl}-11a-methyl-2H,3H,3aH,3bH,4H,5H,9bH,10H,11H-cyclopenta[a]phenanthren-1-one (2)

In a round bottom flask (10 ml), compound **1** (200 mg, 0.58 mmol), 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol), boric acid (50 mg 0.80 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:bencene (4:1) system; yielding 44% of product; m.p. 88-90 ^{o}C; IR (V_{max}, cm^{-1}) 3400, 3360, 3322 and 1712: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.20-2.60 (m, 15H), 3.04 (m, 2H), 3.72 (m, 2H), 3.80 (m, 2H), 6.54 (m, 1H), 6.80 (m, 1H), 6.90 (m, 1H), 7.56 (m, 1H), 7.58 (broad, 3H), 7.66-8.70 (m, 5H) ppm. 13.82, 21.63, 26.02, 27.40, 27.69, 31.72, 35.91, 37.52, 44.53, 46.00, 47.8, 50.62, 50.84, 53.40, 106.32, 112.54, 121.10, 122.73, 123.75, 124.54, 126.27, 127.30, 127.90, 129.12, 131.26, 133.95, 136.52, 136.88, 148.96, 159.80, 163.52, 220.52 ppm. EI-MS m/z: 496.27. Anal. Calcd. for $C_{32}H_{36}N_2O_3$: C, 77.39; H, 7.31; N, 5.64; O, 9.66. Found: C, 77.30; H, 7.24.

Synthesis of 4-[(6-{[(2-{[(2-hydroxynaphthalen-1-yl)methylidene]amino}ethyl)amino]methyl}-11a-methyl-1-oxo-2H,3H,3aH,3bH,4H,5H,9bH,10H,11H-cyclopenta[a] phenanthren-7-yl)oxy] benzonitrile (3)

In a round bottom flask (10 ml), compound **2** (200 mg, 0.40 mmol), potassium carbonate (55 mg, 0.40 mmol) and 10 ml of dimethyl sulfoxide 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 44% of product; m.p. 112-114 ^{o}C; IR (V_{max}, cm^{-1}) 3402, 3360, 3322, 2240 and 1710: 0.90 (s, 3H), 1.20-2.60 (m, 15H), 3.04 (m, 2H), 3.60 (m, 2H), 3.64 (m, 2H), 3.80 (m, 2H), 6.48-6.70 (m, 2H), 6.80 (m, 1H), 6.82 (m, 2H), 7.18 (m, 2H), 7.58-8.44 (m, 5H), 8.70 (d, 1H, J = 1.50 Hz) 8.80 (broad, 2H) ppm. 13.82, 21.62, 23.42, 26.02, 27.41, 27.70, 31.72, 35.92, 37.53, 45.20, 46.00, 47.82, 50.62, 50.82, 53.40, 106.30, 111.92, 117.42, 121.10, 121.80, 122.48, 122.74, 123.26, 123.80, 126.24, 126.34, 127.31, 129.12, 129.30, 129.84, 129.84, 130.86, 133.92, 136.52, 141.40, 152.02, 154.82, 159.80, 163.50, 220.54 ppm. EI-MS m/z: 611.31. Anal. Calcd. for $C_{40}H_{41}N_3O_3$: C, 78.53; H, 6.76; N, 6.87; O, 7.85. Found: C, 78.48; H, 6.70.

Physicochemical properties.

Several electronic parameters such as HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital), molar refractivity, molar volume, polarizability, parachor, index of refraction, surface tension and density were evaluated using ACDLab [15] and SPARTAN´06 program [16].

Pharmacophore evaluation.

The 3D pharmacophore model for the compounds 2, 3 and 4 was determinate using LigandScout 4.08 software [17]

Theoretical evaluation of the interaction between both compounds 2 and 3 with 17β-hydroxysteroid dehydrogenase type 1 (3HB4).

The interaction of compound 4 with 1IOL [18] protein was carried out using the Docking Server software [19]; additionally, two 17β-HD types 1 inhibitors such as fisetin and methyl paraben [20, 21] were used as controls.

3. RESULTS

Some data indicate that several drugs can exert biological activity against the 17β-HD type 1; however, their interaction with this enzyme is very confusing. Therefore, in this investigation, two steroid derivatives were prepared to characterize their interaction with 17β-HD type 1; the first stage was achieved by the synthesis of two steroid derivatives as follows:

Preparation of a steroid-imino-derivative (2.)

There are several reports to synthesis of imino-derivatives using some reagents such as iron or cobalt [22], $ClCH_2COCl/Et_3N$ [23], Iodide [24], $KSCN/Br_2$ [25], HOAc [26] and others. In this study, the estradiol-ethylenediamine reacted with 2-hydroxy-1-naphthaldehyde to form a hydroxynaphthalen-steroid derivative (2).

The ^{1}H NMR spectra for **2** (Figure 1) showed several signals at 0.90 ppm for methyl bound to steroid nucleus; at 1.20-2.60, 6.54 and 6.90 ppm for steroid moiety: at 3.72 ppm for methylene bound to both amino group and ring A of steroid nucleus; at 6.80 and 7.56-8.70 ppm for phenyl groups; at 3.04, 3.80 ppm for methylene groups bound to both amino groups; at 7.58 ppm for both amino and hydroxyl groups. ^{13}C NMR spectra for **2** showed several signals at 13.82 ppm for methyl bound o steroid nucleus; at 21.63-37.52, 46.00-50.62, 112.54, 124.54, 127.90, 131.26, 136.88-148.96 ppm for steroid moiety; at 44.53 ppm for methylene bound to both ring A and amino group; at 50.84-53.40 ppm for methylene groups bound to both amino groups; at 106.32, 121.10-123.75, 126.27-127.30, 129.12, 133.95-136.52 and 159.80 ppm for phenyl groups; at 163.52 ppm for imino group; at 220.52 ppm for ketone group. In addition, compound **2** showed a molecular ion (m/z) at 496.27.

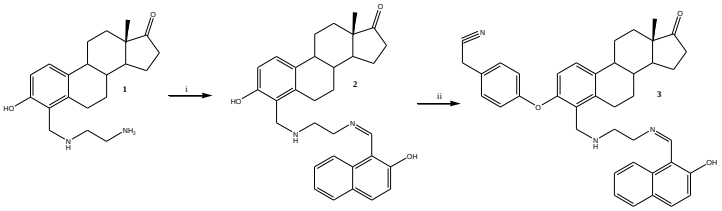

Figure 1. Synthesis of two steroid-derivatives (**2** or **3**). Reaction of estradiol-ethylenediamine (**1**) with 2-hydroxy-1-naphthaldehyde to form a hydroxynaphthalen-steroid derivative (**2**). Then, 2 reacted with (4-Nitro-phenyl)-acetonitrile to synthesis of *a* steroid-naphthalen-methylene-ethylenediamine derivative (**3**).

Preparation of a steroid-naphthalen-methylene-ethylenediamine derivative (3).

Some methods have been reported for preparation of ether derivatives using some reagents such as methoxy groups [27],

fluoride ion [28], nitroparaffin [29], sodium phenoxide [30], nitrobenzamide in DMSO [31] and others. In this regard, compound **2** was reacted with (4-Nitro-phenyl)-acetonitrile (Figure 1) to form an ether-steroid derivative (**3**) using previously reports for preparation of ether groups [32]. The ^{1}H NMR spectra for **3** showed several signals at 0.90 ppm for methyl bound to steroid nucleus; at 1.20-2.60 and 6.48-6.70 ppm for steroid moiety: at 3.04, 3.80 ppm for methylene groups bound to both amino groups; at 3.60 ppm for methylene bound to both amino group and ring A of steroid nucleus; at 3.64 ppm for methylene bound to both phenyl and alkyne groups; at 6.82-8.44 ppm for phenyl groups; at 8.80 ppm for both amino and hydroxyl groups. ^{13}C NMR spectra for **3** showed several signals at 13.82 ppm for methyl bound o steroid nucleus; at 21.62, 26.02-37.53, 46.00-50.62, 111.92, 126.34, 129.30-130.86 and 141.40-151.02 ppm for steroid moiety; at 23.42 ppm for methylene bound to both phenyl and alkyne groups; at 45.20 for methylene bound to both ring A and amino group; at 50.82-53.40 ppm for methylene groups bound to both amino groups; at 106.30, 121.10-126.64, 127.31-129.12, 133.92-136.52 and 154.82-159.80 ppm for phenyl groups; at 117.40 ppm for nitrile; at 163.50 ppm for imino group; at 220.54 ppm for ketone group. Finally, the compound **3** showed a molecular ion (m/z) at 611.31.

Electronic parameters relationship to compounds 2 and 3.

Molecular orbitals HOMO and LUMO for 2 and 3 were evaluated using SPARTAN´06 program, with Hartee-Fock metod at 321-G level [16]. The results showed that both HOMO an LUMO were higher for compound **3** compared with **2** (Figure 2).

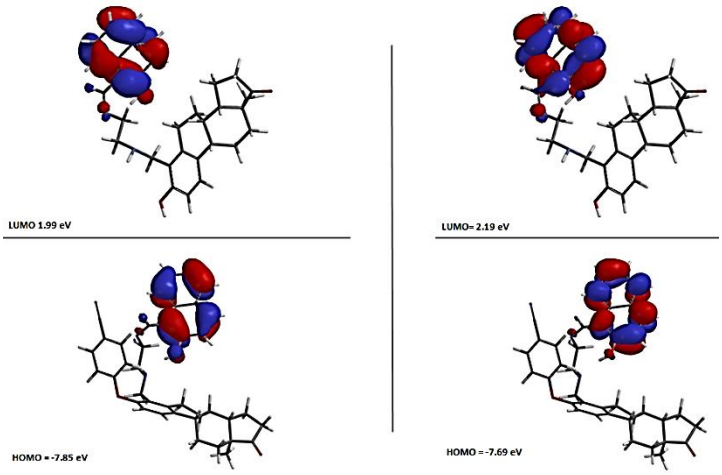

Figure 2. In the scheme are showed both HOMO and LUMO for compounds 2 (left) and 3 (right). Visualized with SPARTAN´06 program.

Table 1. Physicochemical parameters of compounds **2, 3** and **4**. The values were calculated using both ACDLabs and Spartan softwars.

Parameter	Compound 2	Compound 3
Molar Refractivity (cm^3)	143.70	175.91
Molar Volume (cm^3)	381.70	47.30
Polarizability (cm^3)	56.98	69.73
Parachor (cm^3)	1024.90	1261.10
Index of refraction	1.67	1.66
Surface Tension (dyne/cm)	51.90	51.20
Density g/cm^3	1.30	1.26

In addition, other physicochemical parameters showed in the table 1 were evaluated the results indicate that molar refractivity and molar volume were higher for compound 3 compared with 2. These data suggest that steric hindrance, conformational preferences and internal rotation of 3 could influence the interaction with some biomolecules.

Pharmacophore Modeling.

There are some studies that indicate that the pharmacophore is the three-dimensional orientation adopted by the functional groups of a molecule to be able to interact with some proteins [33].

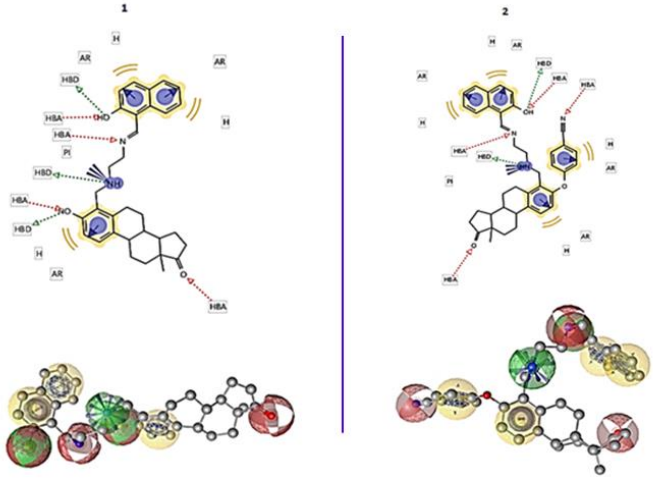

Figure 3. Scheme represents a pharmacophore from both compounds **2** and **3** using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red) and hydrogen bond donor (HBD, green).

This 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. Therefore, in this study, LigandScout software [17] was used to develop a pharmacophore model of compounds **2** and **3**. The results showed in Figure 3 indicated that there is a different type of functional groups involved in the compounds **2** and **3** that can interact via hydrophobic contacts or as hydrogen bond acceptors or as hydrogen bond donor with some biomolecules .

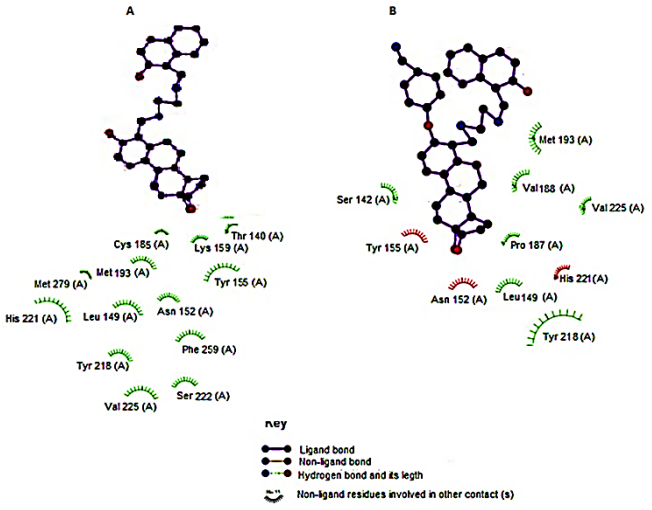

Figure 3. Theoretical analysis showed the binding sites for compounds 2 (A) and 3 (B) with some amino acid residues involved in 17β-HD type 1 (1IOL). The visualization was carried out using the DockingServer software.

Interaction of both compounds 2 and 3 with 17β-HD type 1 (1IOL).

There are reports to evaluate the interaction of several drugs with some biomolecules using theoretical models [34-37]. In this study the interaction of both compounds 2 and 3 with 1IOL enzyme [18] was evaluated using the fisetin and methyl paraben as controls in a Docking model [19]. The theoretical data (Table 2) showed that compound **2** interact with different type of amino acid residues involved in 1IOL surface compared with compound **3,** fisetin and methyl paraben. This phenomenon could involve other type intramolecular interactions due to changes in the energy levels.

Table 2. Aminoacid residues involved in the interaction of fisestin and compounds **2** and **3** with 17β-HD type 1 (3HB4).

----------Compounds----------			
Methyl Paraben	**Fisetin**	**2**	**3**
Leu_{96}	Ser_{142}	Thr_{140}	Ser_{142}
Tyr_{218}	Leu_{149}	Leu_{149}	Leu_{149}
His_{221}	Asn_{152}	Asn_{152}	Asn_{152}
Ser_{222}	Cys_{185}	Tyr_{155}	Tyr_{155}
Val_{225}	Pro_{187}	Lys_{159}	Pro_{187}
Phe_{226}	Val_{188}	Cys_{185}	Val_{188}
Met_{279}	Thr_{190}	Met_{193}	Met_{193}
Glu_{282}	Met_{193}	Tyr_{218}	Tyr_{218}
Val_{283}	Tyr_{218}	His_{221}	His_{221}
	Ser_{222}	Ser_{222}	Val_{225}
		Val_{225}	
		Phe_{259}	
		Met_{279}	

Thermodynamic parameters.

Some studies showed that several thermodynamic parameters may be involved in the interaction drug-protein [38]. Analyzing these data, in this study a theoretical ass was carried out to evaluate several thermodynamic factors involved in the interaction of fisetin, methyl paraben and the compounds **2** and **3** with the 1IOL protein such as 1) free energy of binding which determinate the energy value that require a molecule to interact with a protein in a water environment. 2) Electrostatic energy that is the product of electrical charge and electrostatic potential, which are involved in the ligand-protein system; 3) total intermolecular energy and 4) Van der Waals (vdW) + hydrogen bond (Hbond) + desolvation energy (Desolv. Energy; which have an influence on the movement of water molecules into or out of the ligand-protein system) using a theoretical model [19]. Theoretical data (Table 3) indicates that there are differences in the thermodynamic parameters of compound **2** compared with fisetin, methyl paraben and compound **3**.

Additionally, the inhibition constant (Ki) for compound **2** was lower than Ki for fisetin, methyl paraben and compound **3** (Table 3). This phenomenon suggests that these differences could be translated as a higher inhibition of biological activity of 17β-hydroxysteroid dehydrogenase type 1 **(**1IOL**)** in the presence of compound **2** in comparison with fisetin, methyl paraben and compound **3**.

Table 3. Termodynamic parameters involved in the interaction of fisetin and compounds **2** and **3** with 17β-HD type 1 (3HB4).

Compound	Est. Free Energy of Binding (Kcal/mol)	Est. Inhibition Constant, Ki (μM)	VdW + Hbond +desol Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Total intermolec. Energy (Kcal/mol)	Interact. Surface
Methyl Paraben	-3.55	2.50	-4.25	-0.18	-4.43	461.65
Fisetin	-5.75	60.51	-6.21	-0.10	-6.32	678.67
2	-7.96	1.45	-10.08	-0.19	-10.27	1159.68
3	-9.43	122.14	-11.03	-0.00	-11.03	1255.97

4. CONCLUSIONS

In this study, a facile synthesis of two steroid-derivatives is reported. In addition, theoretical interact suggest that of compound **2** could act as 17β-hydroxysteroid dehydrogenase type 1 inhibitor; this phenomenon could be translated as a good candidate for the treatment of cancer.

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Design and synthesis of two steroid-oxirane-carboxamide derivatives

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Abstract

There are several studies to preparation of oxirane-derivatives which use several reagents such as chlorophyll, ethyl bromoacetate, *m*-chloroperoxybenzoic acid, potassium hydroxide, dimethyldioxiran and others; nevertheless, expensive reagents and special conditions are required. The aim of this study was synthesizing two novel steroid-oxirane using some reactions; the first stage was achieved by the preparation of two steroid-propargylic-ether (**3** or **4**) via reaction of 2-nitroestrone or 2-nitroestradiol with 5-hexyn-1-ol. The second stage involves the synthesis of two steroid-dioxa derivatives (**5** or **6**) via intramolecular addition from **3** or **4** using Copper(II) as catalyst. Then, **5** or **6** were reacted with ethylenediamine to form two steroid-amino derivatives (**7** or **8**). Following, the compounds **9** or **10** were prepared through of reaction of **7** or **8** with chloroacetylchloride. Finally, **9** or **10** reacted with 2-hydroxy-1-naphthaldehyde to synthesis of two oxirane-steroid derivatives (**11** or **12**). The structure of steroid-oxirane analogs was confirmed via spectroscopic and spectrometric methods. In conclusion, a facile procedure for the preparation of two steroid-oxirane derivatives was developed in this study.

Keywords: Steroid, Oxirane, Cycloaddition, Epoxidation, Amidation, Carboxamide.

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1. Introduction

There are studies which show the preparation of several oxirane derivatives using some methods; for example, the synthesis of 2,3-diphenyl-oxirane-2-carboxylic acid methyl ester from a diazo-carbonyl using NHC-Ag+ as catalyst [1]. Other data showed the epoxidation of 4,4-dimethyl-2-pentyn-1-ol with titanium isopropoxide to form (3-tert-Butyl-2-tributylstannanyl-oxiranyl)-methanol [2]. In addition, the 2-isopropenyl-3-phenyl-oxirane was prepared using the three-component system ((2*R*,5*R*)-dimethylthiolane, allyl halides and aldehyde derivative) [3]. Other study showed the synthesis of an oxirane derivative by reaction of 2,2-dibromomethylquinoxaline with an aromatic aldehyde in the presence of the tetrakis(dimethylamino)ethylene reagent [4]. Also, a 9H-fluorene-2-yl keto-oxirane have been synthesized via epoxidation of the compound 9H-fluorene-2-yl chalcone [5]. Additionally, a spiro-indole-3,2′-oxirane derivative was prepared through epoxidation of 3-aroylmethylene-indole-2-one using an ammonium-bromide derivative as catalyst [6]. Other data shown that a 17-steroid-oxirane was prepared by the reaction of estrone with dimethylsulfonium methylide [7]. In addition, a report indicates the epoxidation of 4-methylene-5-cholestan-3-ol with *m*-chloroperoxybenzoic acid to form a steroid-4-oxirane derivative [8]. Recently, a study showed the preparation of an estrone-17-oxirane by reaction of lactone-steroid derivative with 2-benzothiazol-ylchloromethyl-lithium [9]. All these experimental results indicate that some procedures are available for synthesis of several oxirane analogs; nevertheless, expensive reagents and special conditions are required. Therefore, in this study, two oxirane-steroid derivatives were synthesized using some strategies.

2. Experimental

2.1 General methods

The compounds 2-nitro-estrone was prepared using a previously method reported [10]. 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.

2.2 Preparation of steroid-propargylic-ether (3 or 4)

A solution of 2-nitroestrone or 2-nitroestradiol (0.50 mmol), 5-hexyn-1-ol (70 µl; 0.58 mmol), potassium carbonate (40 mg, 0.30 mmol) in 5 ml of dimethyl sulfoxide was stirring to room temperature for 48 h. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water (4:1) system.

8-(hex-5-yn-1-yloxy)-11a-methyl-1H,2H,3H, 3aH,3bH,4H,5H,9bH,10H,11H-cyclopenta[a] phenanthrene-1,7-diol (3)

yielding 44% of product, m.p. 132-134°C; IR (V_{max}, cm^{-1}) 3400, 2160 and 1110. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-1.40 (m, 7H), 1.60 (m, 2H), 1.66-1.80 (m, 3H), 1.86 (m, 2H), 1.88 (m, 1H), 1.94 (s, 1H), 2.12 (m, 1H), 2.24 (m, 2H), 2.50-3.64 (m, 4H), 4.10 (m, 2H), 6.12 (broad, 2H), 6.40-6.60 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.82, 18.02, 24.22, 25.05, 25.35, 27.77, 28.92, 29.68, 32.78, 33.71, 37.28, 44.00, 44.39, 50.72, 68.64, 70.25, 82.44, 84.10, 107.50, 114.04, 128.81, 133.77, 144.70, 144.92 ppm. EI-MS 368.23. Anal. Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75; O, 13.02. Found:C, 78.16; H, 8.70.

8-(hex-5-yn-1-yloxy)-7-hydroxy-11a-methyl-2H,3H,3aH,3bH,4H,5H,9bH,10H,11H-cyclopenta[a]phenan- thren-1-one (4)

yielding 54% of product, m.p. 146-148°C; IR (V_{max}, cm^{-1}) 2162, 1712 and 1112. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.92 (s, 3H), 1.20-1.52 (m, 5H), 1.60 (m, 2H), 1.80 (m, 1H), 1.86 (m, 2H), 1.92 (m, 1H), 1.94 (s, 1H), 2.10-2.20 (m, 4H), 2.24 (m, 2H), 2.46-2.80 (m, 4H), 4.08 (m, 2H), 5.90 (broad, 1H), 6.40-6.60 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C:13.80, 18.02, 21.74, 25.06, 25.87, 26.43, 28.92, 29.66,

31.50, 35.43, 37.53, 46.86, 48.12, 50.42, 68.62, 70.24, 84.12, 107.14, 114.04, 128.42, 133.34, 144.70, 144.91, 220.70 ppm. EI-MS 366.21. Anal. Calcd. for $C_{24}H_{30}O_3$: C, 78.65; H, 8.25; O, 13.10. Found: C, 78.60; H, 8.20.

2.3 Synthesis steroid-dioxecine derivatives

A solution of **3** or **4** (0.50 mmol), Copper(II) chloride anhydrous (40 mg, 0.30 mmol) in 5 ml of methanol was stirring to room temperature for 48 h**.** The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (4:1:1) system.

18-methyl-4,11-dioxapentacyclo[12.11.0.0^{3,12}.0^{15,23}.0^{18,22}]pentacosa-1,3(12),13-trien-5-yn-19-ol (5)

yielding 45% of product, m.p. 162-164^{o}C; IR (V_{max}, cm^{-1}) 3402, 2192 and 1112. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-1.11 (m, 4H), 1.18 (m, 2H), 1.30-1.40 (m, 3H), 1.60 (m, 2H), 1.66-1.88 (m, 4H), 1.94-1.96 (m, 2H), 2.12-3.64 (m, 5H), 4.16-4.17 (m, 2H), 6.32 (m, 1H), 6.40 (broad, 2H), 6.60 (m, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.82, 17.20, 24.22, 25.32, 27.74, 29.67, 29.79, 32.16, 32.78, 33.71, 37.28, 44.02, 44.39, 50.76, 51.94, 67.96, 76.72, 82.46, 109.33, 111.61, 129.78, 134.05, 144.52, 147.30 ppm. EI-MS 366.21. Anal. Calcd. for $C_{24}H_{30}O_3$: C, 78.65; H, 8.25; O, 13.10. Found: C, 78.62; H, 8.20.

18-methyl-4,11-dioxapentacyclo[12.11.0.0^{3,12}.0^{15,23}.0^{18,22}]pentacosa-1,3(12),13-trien-5-yn-19-one (6)

yielding 63% of product, m.p. 128-130^{o}C; IR (V_{max}, cm^{-1}) 21.90, 1712 and 1110. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.92 (s, 3H), 1.18 (m, 2H), 1.20-1.52 (m, 5H), 1.60 (m, 2H), 1.80-1.92 (m, 2H), 1.95-1.96 (m, 2H), 2.10-2.80 (m, 8H), 4.16-4.17 (m, 2H), 6.30-6.66 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 13.80, 17.20, 21.75, 25.87, 26.33, 29.67, 29.79, 31.50, 32.14, 35.43, 37.56, 46.87, 48.11, 50.40, 51.94, 67.97, 76.70, 108.96, 111.61, 133.61, 144.50, 147.30, 220.70 ppm. EI-MS 364.20. Anal. Calcd. for $C_{24}H_{28}O_3$: C, 79.09; H, 7.74; O, 13.17. Found: C, 79.00; H, 7.70.

2.4 Preparation of amino-steroid-dioxecine derivative

A solution of **5** (0.50 mmol), ethylenediamine (50 µl, 0.74 mmol) in 5 ml of formaldehyde was stirring to reflux for 12 h**.** The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (4:1:1) system.

2-{[(2-aminoethyl)amino]methyl}-18-methyl-4,11-dioxapentacyclo[12.11.0.0^{3,12}.0^{15,23}.0^{18,22}] pentacosa-1,3(12),13-trien-5-yn-19-ol (7)

yielding 45% of product, m.p. 150-152^{o}C; IR (V_{max}, cm^{-1}) 3400, 3380 and 2190. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-1.16 (m, 4H), 1.18 (m, 2H), 1.30-1.40 (m, 3H), 1.60 (m, 2H), 1.70-1.88 (m, 10H), 1.95-1.96 (m, 2H), 2.10-2.52 (m, 4H), 2.66-2.80 (m, 4H), 3.60 (broad, 4H), 3.64 (m, 1H), 3.70 (m, 2H), 4.16-4.17 (m, 2H), 6.36 (m, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.80, 17.20, 24.22, 25.34, 27.69, 27.70, 29.80, 32.16, 32.78, 33.71, 37.28, 41.57, 44.40, 44.60, 46.10, 50.76, 51.95, 53.32, 67.96, 78.38, 82.46, 109.76, 128.75, 132.11, 132.13, 141.32, 143.30 ppm. EI-MS 438.28. Anal. Calcd. for $C_{27}H_{38}N_2O_3$: C, 73.94; H, 8.73; N, 6.39; O, 10.94. Found:C, 73.88; H, 8.68.

2.5 Reduction of hydroxyl from 7

A solution of 7 (0.50 mmol), pyridinium chlorochromate (100 mg, 0.46 mmol) in 5 ml ethanol:water (4:1) at room temperature for 48 h. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (4:2:1) system.

2-{[(2-aminoethyl)amino]methyl}-18-methyl-4,11-dioxapentacyclo[12.11.0.0^{3,12}.0^{15,23}.0^{18,22}]pentacosa-1,3(12),13-trien-5-yn-19-one (8)

yielding 53% of product, m.p. 191-193; IR (V_{max}, cm^{-1}) 3380, 2190, 1710 and 1112. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.18 (m, 2H), 1.20-1.54 (m, 5H), 1.60 (m, 2H), 1.80-1.92 (m, 2H), 1.95-1.96 (m, 2H), 2.10-2.54 (m, 8H), 2.64 (m, 2H), 2.66 (broad, 3H), 2.80-3.70 (m, 4H), 4.16-4.17 (m, 2H), 6.44 (m, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 13.82, 17.20, 21.72, 25.72, 27.41, 27.70, 29.79, 31.32, 32.16, 35.12, 37.50, 41.56, 46.10, 47.44, 48.22, 50.54, 51.95, 53.32, 67.96, 78.38, 109.39, 128.38, 131.69, 132.11, 141.32, 143.30, 220.70 ppm. EI-MS 436.27. Anal. Calcd. for $C_{27}H_{36}N_2O_3$: C, 74.28; H, 8.31; N, 6.42; O, 10.99. Found:C, 74.20; H, 8.26.

2.6 Synthesis of chloroamide derivative

A solution of **7** or **8** (0.50 mmol), chloroacetyl chloride (50 µl, 0.63 mmol) and triethylamine (80

µl, 0.57 mmol) in 5 ml methanol at room temperature for 48 h**.** The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:ketone (3:1) system.

2-chloro-N-{2-[({19-hydroxy-18-methyl-4,11-dioxapentacyclo[12.11.0.0^{3,12}.0^{15,23}.0^{18,22}]penta-cosa-1,3(12),13-trien-5-yn-2-yl}methyl)amino] ethyl}acetamide (9)

yielding 83% of product, m.p. 166-168^{o}C; IR (V_{max}, cm^{-1}) 3400, 3310, 2190 and 1110. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-1.16 (m, 4H), 1.18 (m, 2H), 1.30-1.40 (m,3H), 1.60 (m, 2H), 1.70-1.88 (m, 4H), 1.95-1.96 (m, 2H), 2.10-2.52 (m, 4H), 2.70-3.40 (m, 4H), 3.64 (m, 1H), 3.70 (m, 2H), 4.02 (m, 2H), 4.16-4.17 (m, 2H), 5.94 (broad, 3H), 6.36 (m, 1H). ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.80, 17.20, 24.22, 25.35, 27.70, 27.72, 29.80, 32.16, 32.78, 33.71, 37.28, 38.57, 42.43, 44.40, 44.62, 46.10, 50.76, 51.95, 67.96, 78.38, 82.46, 109.76, 128.75, 132.13, 132.37, 141.34, 143.30, 162.60 ppm. EI-MS 514.25. Anal. Calcd. for $C_{29}H_{39}ClN_2O_4$: C, 67.62; H, 7.63; Cl, 6.88; N, 5.44; O, 12.42. Found: C, 67.60; H, 7.58.

2-chloro-N-{2-[({18-methyl-19-oxo-4,11-dioxa-pentacyclo[12.11.0.0^{3},^{12}.0^{15,23}.0^{18,22}]pentacosa-1,3(12),13-trien-5-yn-2-yl}methyl)amino]ethyl} acetamide (10)

yielding 78% of product, m.p. 154-156^{o}C; IR (V_{max}, cm^{-1}) 3310, 21.90, 1712 and 1112. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.18 (m, 2H), 1.20-1.54 (m, 5H), 1.60 (m, 2H), 1.80-1.92 (m, 2H), 1.95-1.96 (m, 2H), 2.10-2.54 (m, 8H), 2.70-3.40 (m, 4H), 3.70 (m, 2H), 4.02 (m, 2H), 4.16-4.17 (m, 2H), 5.76 (broad, 2H), 6.44 (m, 1H). ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 13.82, 17.20, 21.72, 25.72, 27.41, 27.70, 29.80, 31.33, 32.16, 35.12, 37.50, 38.57, 42.40, 46.10, 47.45, 48.21, 50.54, 51.94, 52.84, 67.96, 78.38, 109.39, 128.38, 131.69, 132.37, 141.34, 143.32, 162.60, 220.70 ppm. EI-MS 512.24. Anal. Calcd. for $C_{29}H_{37}ClN_2O_4$: C, 67.89; H, 7.27; Cl, 6.91; N, 5.46; O, 12.47. Found:C, 67.80; H, 7.20.

2.7 Preparation of chloroamide derivative

A solution of **9** or **10** (0.50 mmol), 2-hydroxy-1-naphthaldehyde (68 mg, 0.40 mmol), and sodium hydroxide (20 mg, 0.50 mmol) in 5 ml of ethanol was stirring for 72 h at room temperature. 2-hydroxy-1- naphthaldehyde (68 mg, 0.40 mmol), and sodium hydroxide (20 mg, 0.50 mmol) in 5 ml of ethanol was stirring for 72 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water (4:1) system.

N-{2-[({19-hydroxy-18-methyl-4,11-dioxapenta cyclo[12.11.0.0^{3,12}.0^{15,23}.0^{15,23}.0^{18,22}]pentacosa-1,3 (12),13-trien-5-yn-2-yl}methyl)amino]ethyl}-3-(2-hydroxy-naphthalen-1-yl)oxirane-2-carbo-xamide (11)

yielding 54% of product, m.p. 175-177^{o}C; IR (V_{max}, cm^{-1}) 3402, 2192, 1632 and 1112. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-1.16 (m, 4H), 1.18 (m, 2H), 1.30-1.40 (m,3H), 1.60 (m, 2H), 1.70-1.88 (m, 4H), 1.95-1.96 (m, 2H), 2.10-2.52 (m, 4H), 2.70-3.40 (m, 4H), 3.64 (m, 1H), 3.70 (m, 2H), 3.94 (m, 1H), 4.16-4.17 (m, 2H), 4.26 (m, 1H), 6.36 (m, 1H), 6.66 (broad, 4H), 7.22-7.90 ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.80, 17.20, 24.22, 25.35, 27.69, 27.70, 29.80, 32.16, 32.78, 33.71, 37.30, 39.14, 44.39, 44.60, 46.04, 50.76, 51.94, 52.82, 53.66, 59.55, 67.94, 78.38, 82.46, 109.76, 118.80, 121.45, 122.58, 123.43, 126.81, 128.00, 128.75, 129.2, 130.35, 132.13, 132.37, 134.34, 141.30, 143.33, 152.77, 172.20 ppm. EI-MS 650.33. Anal. Calcd. for $C_{40}H_{46}N_2O_6$: C, 73.82; H, 7.12; N, 4.30; O, 14.75. Found:C, 73.78; H, 7.08.

3-(2-hydroxynaphthalen-1-yl)-N-{2-[({18-methyl-19-oxo-4,11-dioxapentacyclo[12.11.0.0^{3},^{12}.0^{15,23}.0^{18,22}]penta- cosa-1,3(12),13-trien-5-yn-2-yl}methyl)amino]ethyl}-oxirane-2-carboxamide (12)

yielding 38% of product, m.p. 164-166; IR (V_{max}, cm^{-1}) 21.90, 1712, 1630 and 1110. ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.18 (m, 2H), 1.20-1.54 (m, 5H), 1.60 (m, 2H), 1.80-1.92 (m, 2H), 1.95-1.96 (m, 2H), 2.10-2.54 (m, 8H), 2.70-3.40 (m, 4H), 3.70 (m, 2H), 3.96 (m, 1H), 4.16-4.17 (m, 2H), 4.26 (m, 1H), 6.44 (m, 1H), 6.70 (broad, 3H), 7.22-7.90 (m, 6H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 13.82, 17.20, 21.72, 25.72, 27.41, 27.70, 29.79, 31.30, 32.16, 35.12, 37.49, 39.16, 46.10, 47.45, 48.2 , 50.52, 51.96, 52.84, 53.66, 59.55, 67.96, 78.38, 109.40, 118.80, 121.45, 122.58, 123.43, 126.81, 128.00, 128.38, 129.2, 130.35, 131.70, 132.37, 134.34, 141.30, 143.34, 152.74, 172.20, 220.72 ppm. EI-MS 648.31. Anal. Calcd. for

$C_{40}H_{44}lN_2O_6$: C, 74.05; H, 6.84; N, 4.32; O, 14.80. Found:C, 74.00; H, 6.80.

3. Results and Discussion

Several studies have been showed the preparation of oxirane derivatives; however, some protocols use expensive reagents and their management requires special conditions [11-13]. Therefore, in this study, we report a facile method for the synthesis of two steroid-oxirane derivatives using several strategies.

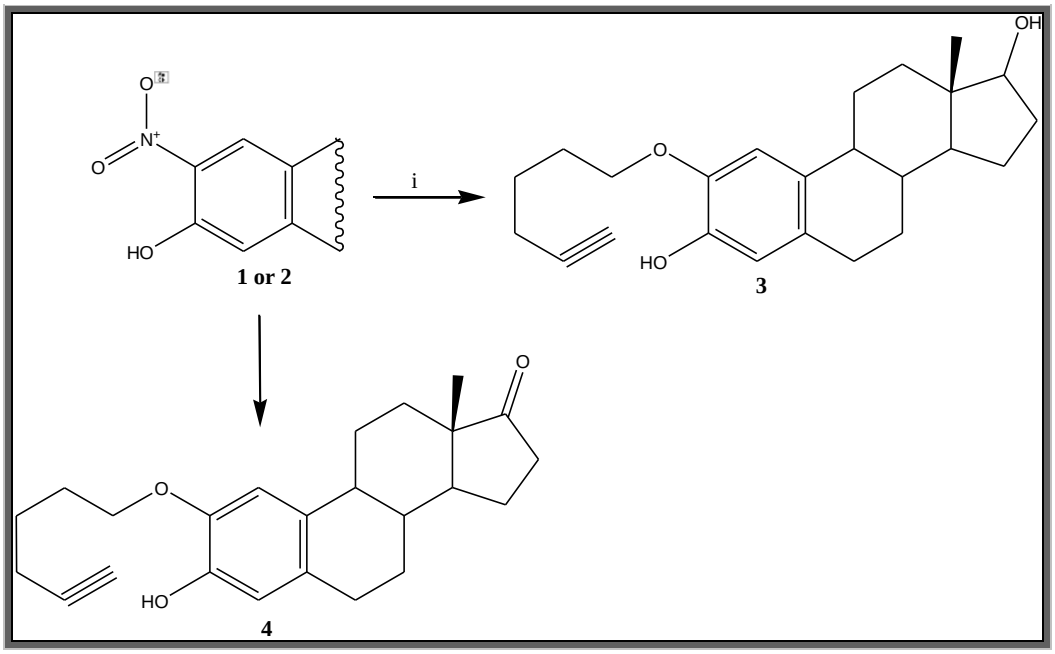

Figure 1. Preparation of steroid-propargylic-ether (**3** or **4**). Reaction of 2-nitroestradiol (**1**) or 2-nitroestrone (**2**) with 5-hexyn-1-ol (i) to form **3** or **4**.

3.1 Preparation of propargyl-ether derivatives

There are several methods for preparation of ether derivatives which involve the use of different reagents such hexyl bromide/sodium cyanide [14], *m*-chloroperoxybenzoic acid [15], hydrazonyl chloride [16], N,N-dimethylbarbituric acid [17] and others. In this study, estrone or estradiol were reacted with 5-hexyn-1-ol in presence of dimethyl sulfoxide at mild conditions.

The ^{1}H NMR spectrum of **3** showed several signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.80-1.40, 1.66-1.80, 1.88, 2.12, 2.50-3.64 and 6.40-6.60 ppm for steroid moiety; at 1.60, 1.86, 2.24 and 4.10 ppm for methylene groups involved in the arm bound of both ether and alkyne groups; at 1.94 ppm for alkyne group; at 6.12 ppm for both hydroxyl groups. The ^{13}C NMR spectra display several chemical shifts at 15.82 ppm for methyl group bound to steroid nucleus; at 18.02, 25.05, 28.92 and 70.25 ppm for methylene groups o arm bound to both ether and alkyne groups; at 24.22, 23.35-27.77, 29.68-50.72, 82.44 and 107.50-144.92 ppm for steroid moiety, at 68.64 and 84.10 ppm for alkyne group. In addition, the mass spectrum from **3** showed a molecular ion (m/z) 368.23.

Other data showed several signals involved in ^{1}H NMR spectrum of compound **4** at 0.90 ppm for methyl group bound to steroid nucleus; at 1.20-1.52, 1.80, 1.92, 2.10-2.20, 2.46-2.80 and 6.40-6.60 ppm for steroid moiety; at 1.60, 1.86, 2.24 and 4.08 ppm for methylene groups involved in the arm bound of both ether and alkyne groups; at 1.94 ppm for alkyne group; at 5.90 ppm for hydroxyl group. The ^{13}C NMR spectra display several chemical shifts at 13.80 ppm for methyl group bound to steroid nucleus; at 18.02, 25.06, 28.92 and 70.24 ppm for methylene groups of arm bound to both ether and alkyne groups; at 21.74, 25.87-26.43, 29.66-50.42 and 107.14-144.91ppm for steroid moiety, at 68.64 and 84.12 ppm for alkyne group; at 220.70 ppm for ketone group.

Finally, the mass spectrum from **4** showed a molecular ion (m/z) 366.21.

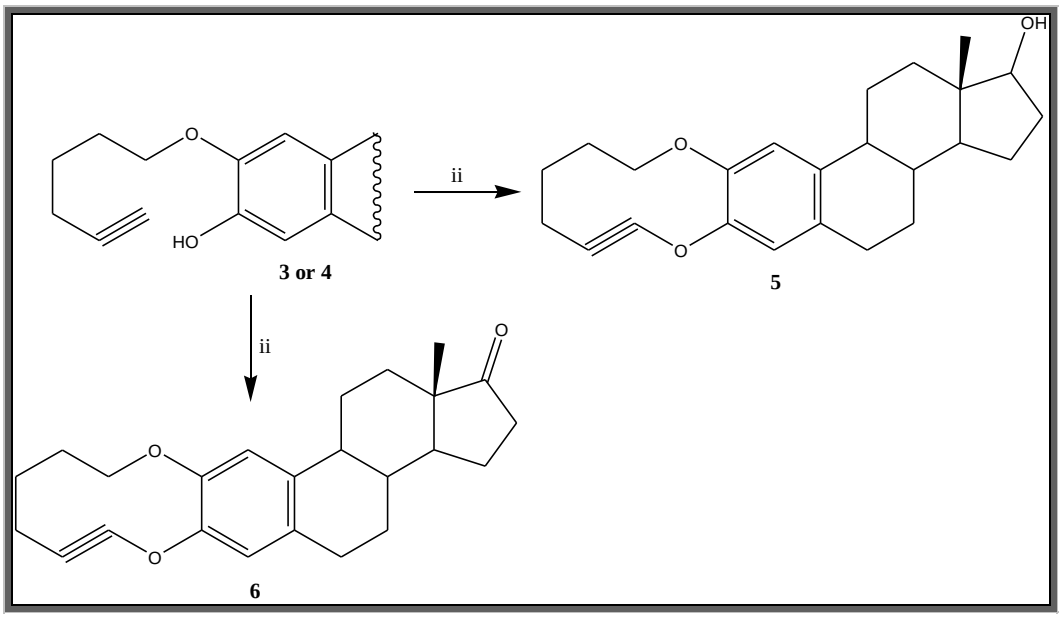

Figure 2. Synthesis of two steroid-dioxecine derivatives (**5** or **6**). Intramolecular reaction of alkyne with the hydroxyl group from 3 or **4** to form the compounds **5** or **6**. ii = Copper(II).

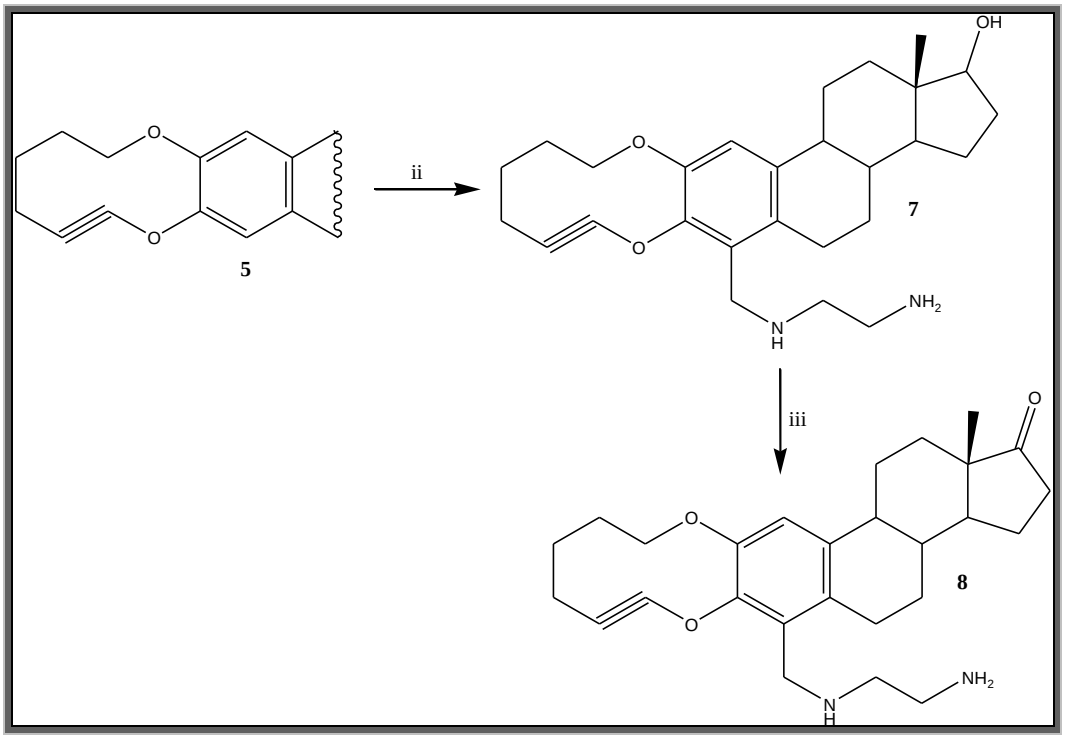

Figure 3. Preparation of two amino-steroid analogs (**7** or **8**). Reaction of oxacine-steroid derivatives (**5**) with ethylenediamine in presence of formaldehyde (ii) to form an amino-ozacine steroid (**7**). Then, **8** was prepared via reduction of **7** with cyanoborohydride/Zn^{+2} (iii).

3.2 Synthesis steroid-dioxa derivatives

There are several reports on the preparation of oxacyclic derivatives using several reagents such as Grubbs catalyst™ 2nd generation [18] urea [19], oxoborane [20], phosphorus trichloride [21], di-ethanolamine [22], *p*-Toluenesulfonamide [23] and others. In this study, two steroid-dioxa

derivatives (**5** or **6**) were prepared via intramolecular from 3 or 4 using Copper(II) as a catalyst (Figure 2); here, is important to mention that this method does not require special conditions.

The ^{1}H NMR spectrum of **5** showed several signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.80-1.11, 1.30-1.40, 1.66-1.88, 2.12-3.64, 6.32 and 6.60 ppm for steroid moiety; at 1.18, 1.60, 1.94-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 6.40 ppm for hydroxyl group. The ^{13}C NMR spectra display several chemical shifts at 15.82 ppm for methyl group bound to steroid nucleus; at 17.20, 29.79-32.16 and 67.96 ppm for dioxecine ring; at 24.22, 29.79-32.16, 50.76 and 82.46-147.30 ppm for steroid moiety, at 52.95-76.72 ppm for alkyne group. In addition, the mass spectrum from **5** showed a molecular ion (m/z) 366.21.

The ^{1}H NMR spectrum of **6** showed several signals at 0.92 ppm for methyl group bound to steroid nucleus; at 1.20-1.52, 1.80-1.92, 2.10-2.80 and 6.30-6.66 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 6.40 ppm for hydroxyl group. The ^{13}C NMR spectra display several chemical shifts at 13.80 ppm for methyl group bound to steroid nucleus; at 17.20, 29.79, 32.14 and 67.97 ppm for dioxecine ring; at 21.75-29.67, 31.50, 35.43-50.40 and 108.96-147.30 ppm for steroid moiety, at 51.94-76.70 ppm for alkyne group. In addition, the mass spectrum from **6** showed a molecular ion (m/z) 364.20.

3.3 Synthesis of steroid-amino derivative

There are several studies that show the preparation of some steroid-amino derivatives with Mannich reaction [24]; these reports indicate the reactivity of hydrogen atom (C-4) involved in ring A of steroid nucleus which can be a specific site to introduce an amino group. Therefore, in this study **5** or **6** were reacted with ethylenediamine in presence of formaldehyde to form two steroid-amino derivatives (**7** or **8**).

The ^{1}H NMR spectrum of **7** showed several signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.80-1.16, 1.30-1.40, 1.70-1.88, 2.10-2.52, 3.64 and 6.36 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 2.66-2.80 ppm for methylene groups bound to both amine groups; 3.60 ppm for both hydroxyl and amino groups; 3.70 ppm for methylene group bound to both ring A (steroid nucleus) and amino group.

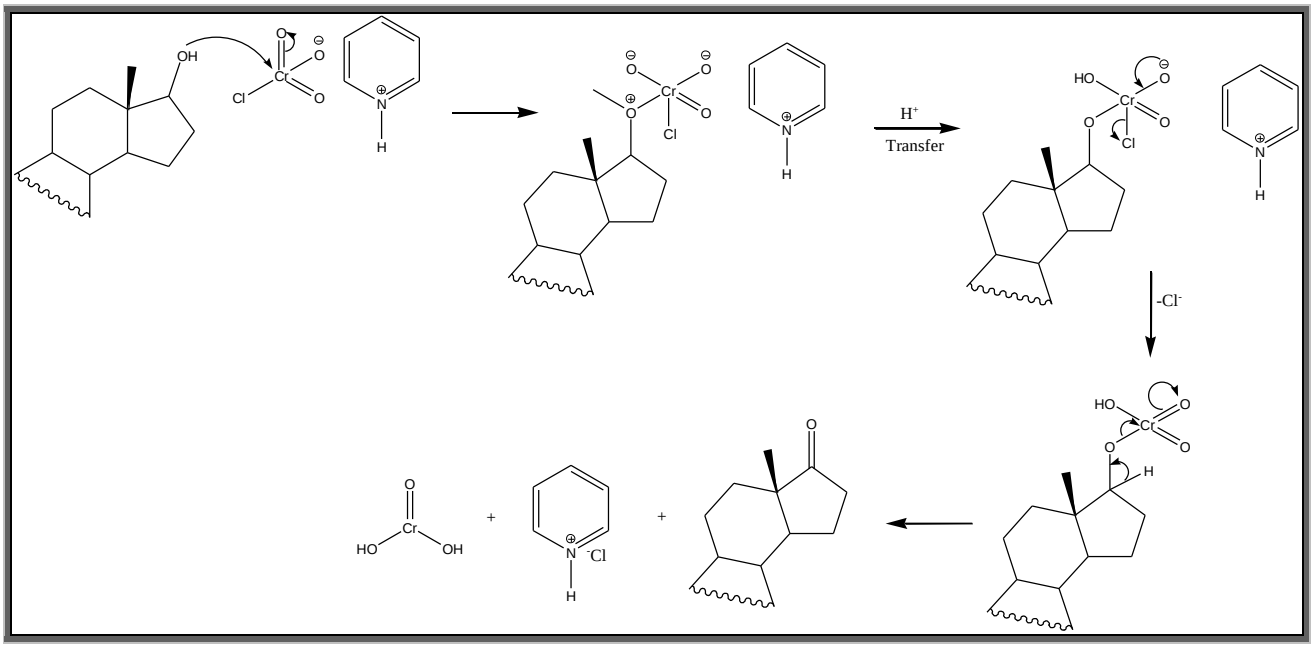

Figure 4. Reaction mechanism involved in the synthesis of an oxacine-steroid derivative (compound **8**) via oxidation of **7** with pyridinium chlorochromate.

The ^{13}C NMR spectra display several chemical shifts at 15.80 ppm for methyl group bound to steroid nucleus; at 17.20, 29.80-32.16 and 67.96 ppm for dioxecine ring; at 25.34-27.70, 32.78-37.28, 44.40-46.00, 50.76 and 82.46-143.30 ppm for steroid moiety, at 41.57 and 53.32 ppm for methylene groups bound to both amino groups; at 46.10 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 51.95 and 78.38 ppm for alkyne group. In addition, the mass spectrum from **7** showed a molecular ion (m/z) 438.28.

Other data showed several signals involved in ^{1}H NMR spectrum for compound **8** at 0.90 ppm for methyl group bound to steroid nucleus; at 1.20-1.54, 1.80-1.92, 2.10-2.54 and 6.44 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 2.64-2.80 ppm for methylene groups bound to both amine groups; 2.66 ppm for amino groups; 3.70 ppm for methylene group bound to both ring A (steroid nucleus) and amino group;. The ^{13}C NMR spectra display several chemical shifts at 13.82 ppm for methyl group bound to steroid nucleus; at 17.20, 29.79, 32.16 and 67.96 ppm for dioxecine ring; at 21.72-27.70, 31.32, 35.12-37.50, 47.44-50.54 and 109.39-143.30 ppm for steroid moiety, at 41.56 and 53.32 ppm for methylene groups bound to both amino groups; at 46.10 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 51.95 and 78.38 ppm for alkyne group; at 220.70 ppm for ketone group. Additionally, the mass spectrum from **8** showed a molecular ion (m/z) 436.27.

3.4 Preparation of steroid-chloroamide derivatives

Several procedures for synthesis of chloroamide derivatives are reported; these protocols involved some reagents such as trichloroisocyanuric Acid [25], *N*-chlorobenzotriazole [26], chloroacetyl chloride [27-29]. Therefore, in this study two steroid-chloroamide derivatives (**9** or **10**) were prepared using chloroacethyl chloride/triethyl-amine; it is important to mention that with this method the yielding was relatively good.

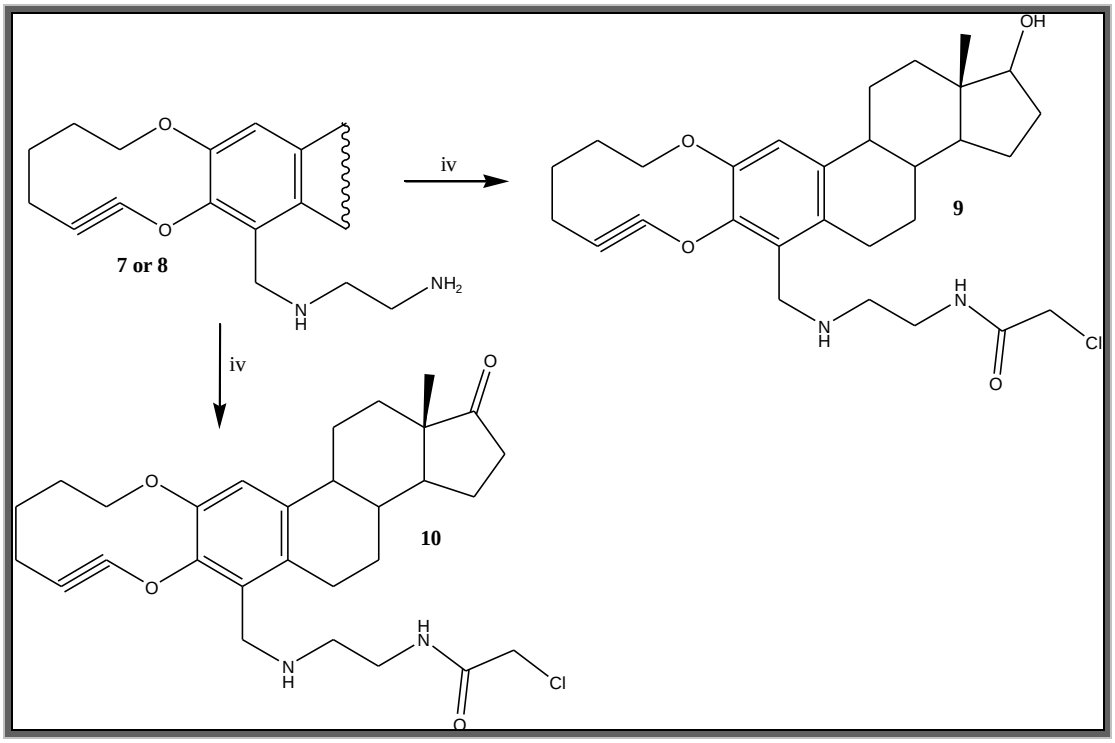

Figure 5. Synthesis of two chloroamide derivatives (**9** or **10**). Compounds **7** o **8** reacted with chloroacetyl chloride (iv) to form **9** or **10**.

The ^{1}H NMR spectrum of **9** showed several signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.80-1.16, 1.30-1.40, 1.70-1.88, 2.10-2.52, 3.64 and 6.36 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine

ring; at 2.70-3.40 ppm for methylene groups bound to both amine groups; 3.70 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 4.02 ppm for methylene bound chloride; at 5.94 ppm for hydroxyl, amino and amide groups. The ^{13}C NMR spectra display several chemical shifts at 15.80 ppm for methyl group bound to steroid nucleus; at 17.20, 29.80-32.16 and 67.96 ppm for dioxecine ring; at 24.22-27.72, 32.7837.28, 44.40-44.62, 50.76 and 82.46-143.30 ppm for steroid moiety, at 38.57 and 52.84 ppm for methylene groups bound to both amino groups; at 42.43 ppm for methylene group bound to chloride; at 46.10 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 51.95 and 78.38 ppm for alkyne group; at 162.60 ppm for amide group. In addition, the mass spectrum from **9** showed a molecular ion (m/z) 514.25.

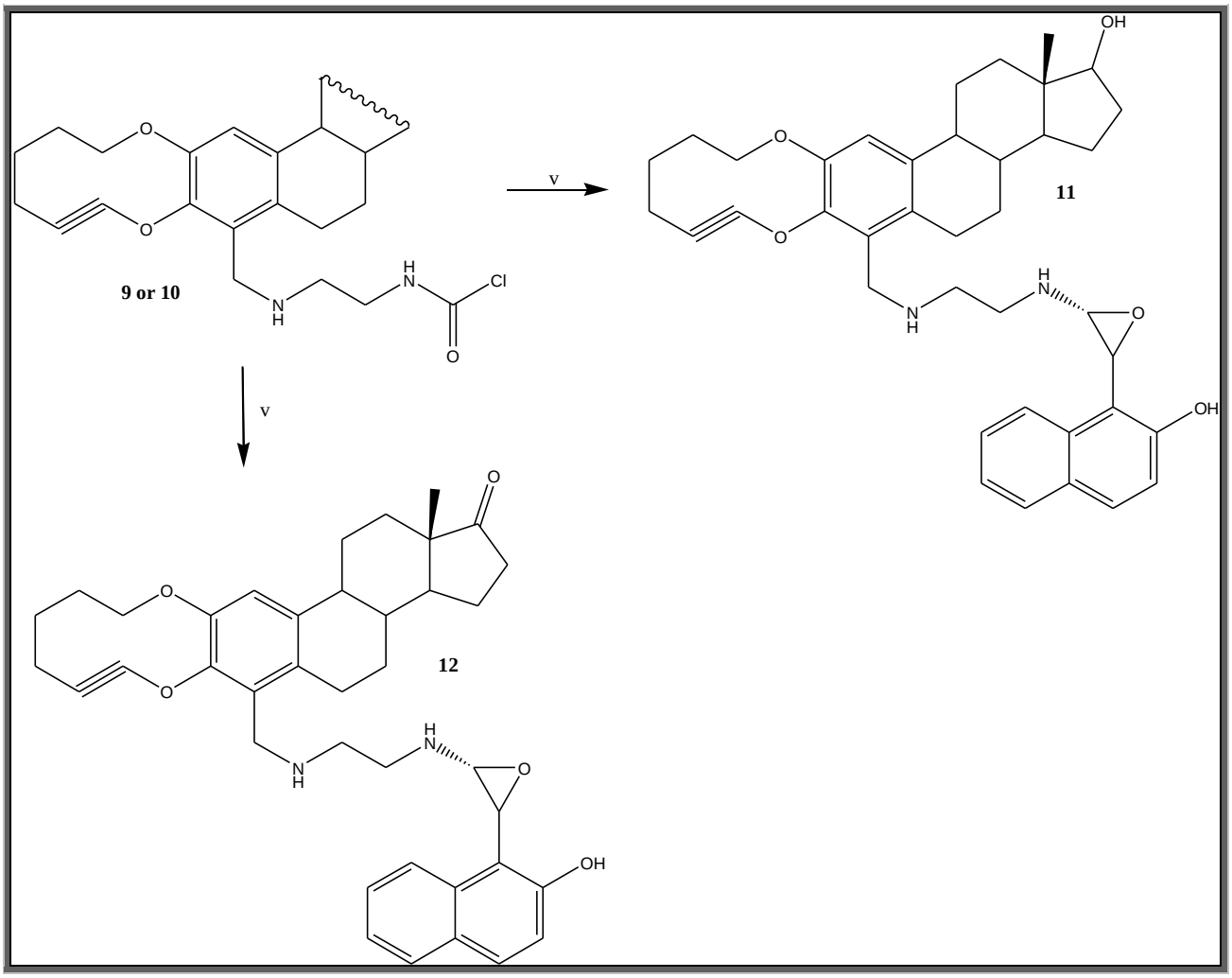

Figure 6. Synthesis of two oxirane-steroid derivatives (**11** or **12**). Compounds **9** or **10** reacted with 2-hydroxy-1-naphthaldehyde (v) to form **11** or **12**.

Other data showed several signals involved in ^{1}H NMR spectrum for the compound **10** at 0.90 ppm for methyl group bound to steroid nucleus; at1.20-1.54, 1.80-1.92, 2.10-2.54 and 6.44 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 2.70-3.40 ppm for methylene groups bound to both amine groups; 3.70 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 4.02 ppm for methylene bound chloride; at 5.76 ppm for both amino and amide groups. The ^{13}C NMR spectra display several chemical shifts at 13.82 ppm for methyl group bound to steroid nucleus; at 17.20, 29.80-32.16 and 67.96 ppm for dioxecine ring; at 21.72-27.70, 31.33, 35.12-37.50, 47.45-50.54 and

109.39-143.32 ppm for steroid moiety, at 38.57 and 52.84 ppm for methylene groups bound to both amino groups; at 42.40 ppm for methylene group bound to chloride; at 46.10 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 51.94 and 78.38 ppm for alkyne group; at 162.60 ppm for amide group; at 220.70 for ketone group. In addition, the mass spectrum from **10** showed a molecular ion (m/z) 512.24.

3.5 Preparation of oxirane-steroid derivatives

Several studies have been reported for synthesis of oxirane derivatives which involve some reagents such as chlorophyll [30], ethyl bromoacetate [31], *m*-chloroperoxybenzoic acid [32], potassium hydroxide [33], dimethyldioxiran [34] and others. In this study, the compounds **9** or **10** were reacted with 2-hydroxy-1-naphthaldehyde in basic medium to form two oxirane-steropid deivatives (**11** or **12**). The ^{1}H NMR spectrum of **11** showed several signals at 0.76 ppm for methyl group bound to steroid nucleus; at 0.80-1.16, 1.30-1.40, 1.70-1.88, 2.10-2.52, 3.64 and 6.36 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 2.70-3.40 ppm for methylene groups bound to both amine groups; 3.70 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 3.94 and 4.26 for oxirane ring; at 6.66 ppm for hydroxyl, amide and amino groups; at 7.22-7.90 ppm for naphthalene. The ^{13}C NMR spectra display several chemical shifts at 15.80 ppm for methyl group bound to steroid nucleus; at 17.20, 29.80-32.16 and 67.94 ppm for dioxecine ring; at 24.22-27.70, 32.78-37.30, 44.39-44.60, 50.76 and 82.46-109.76, 128.75, 132.13-132.37 and 141.30-143.32 ppm for steroid moiety; at 39.14 and 52.82 ppm for methylene groups bound to both amino groups; at 46.04 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 51.94 and 78.38 ppm for alkyne group; at 55.66 and 59.55 ppm for oxirane ring; at 118.80-128.00, 129.20-130.35 and 134.34-152.77 for naphthalene. In addition, the mass spectrum from **11** showed a molecular ion (m/z) 650.33.

Finally, the ^{1}H NMR spectrum of **12** showed several signals at 0.90 ppm for methyl group bound to steroid nucleus; at 1.20-1.54, 1.80-1.92, 2.10-2.54 and 6.44 ppm for steroid moiety; at 1.18, 1.60, 1.95-1.96 and 4.16-4.17 ppm for methylene groups involved in dioxecine ring; at 2.70-3.40 ppm for methylene groups bound to both amine groups; 3.70 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 3.96 and 4.26 for oxirane ring; at 6.70 ppm for hydroxyl, amide and amino groups; at 7.22-7.90 ppm for naphthalene.

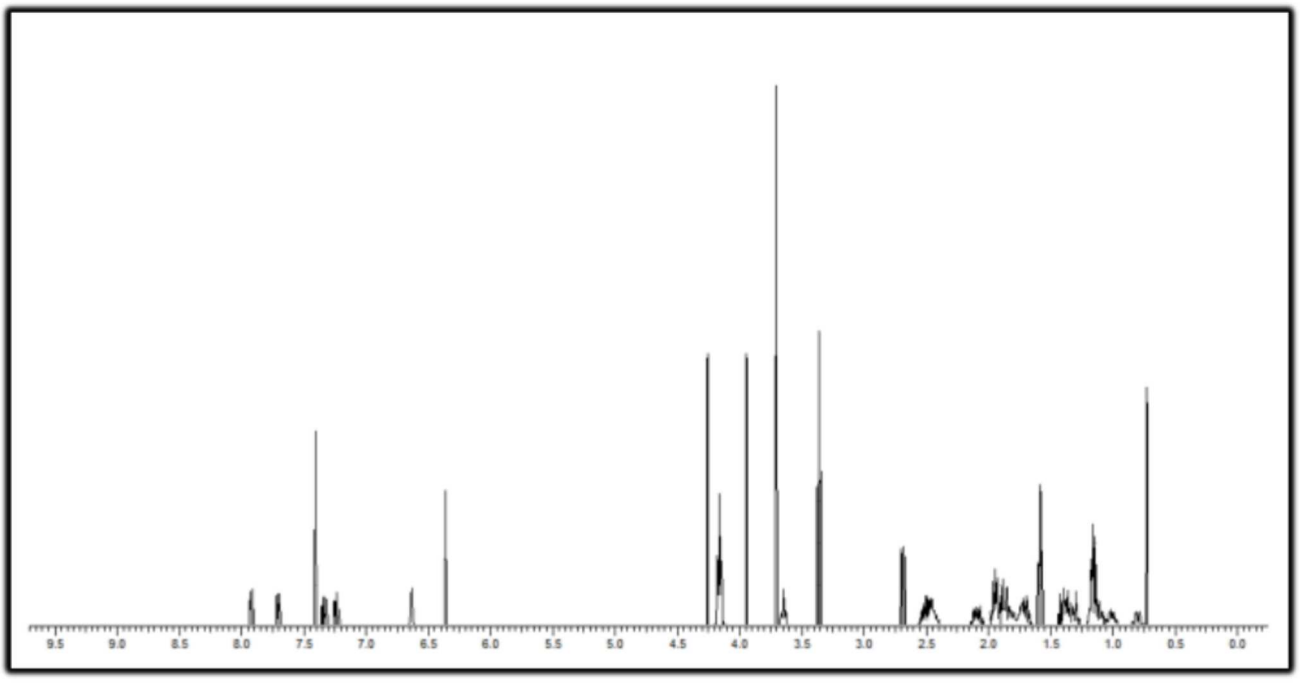

Figure 7. The scheme shown ^{1}H NMR spectrum from **11.** Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in $CDCl_3$. Axis abscissa (ppm); ppm = parts per million.

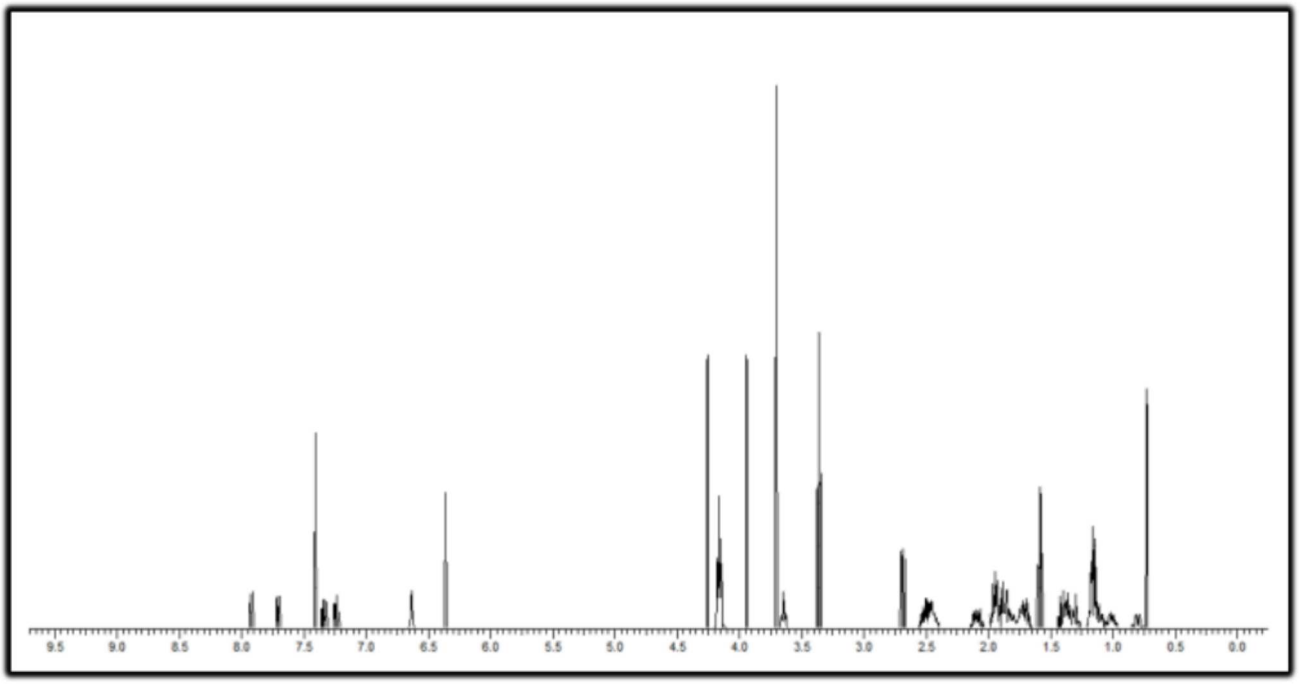

Figure 8. Visualization of ^{1}H NMR spectrum from **12.** Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in $CDCl_3$. Axis abscissa (ppm); ppm = parts per million.

The ^{13}C NMR spectra display several chemical shifts at 13.82 ppm for methyl group bound to steroid nucleus; at 17.20, 29.79, 32.16 and 67.96 ppm for dioxecine ring; at 21.72-27.70, 31.30, 35.12-37.49, 47.45-50.52, 109.40, 128.38, 131.70-132.37 and 141.34-143.30 ppm for steroid moiety; at 39.16 and 52.84 ppm for methylene groups bound to both amino groups; at 46.10 ppm for methylene group bound to both ring A (steroid nucleus) and amino group; at 51.96 and 78.38 ppm for alkyne group; at 55.66 and 59.55 ppm for oxirane ring; at 118.80-128.00, 129.22, 130.35, 134.34 and152.74 for naphthalene; at 172.20 ppm for amide group; at 220.70 ppm for ketone group. In addition, the mass spectrum from **12** showed a molecular ion (m/z) 648.31.

Conclusions

There are several reports for the preparation of oxirane derivatives; however, some protocols use some reagents that can be *i*) expensive; *ii*) difficult to handle; *iii*) expansive and *iv*) require special conditions. Therefore, in this study is reported a facile method for the preparation of two oxiran-steroid derivatives using some strategies. It is important to mention that reagent used for their preparation was not require special conditions and are facile of handled.

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UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO

INSTITUTO DE INVESTIGACIÓN CIENTÍFICA

"Dr. Roberto Rivera Damm"

Otorga la presente:

Constancia

Al C. Dr. Abelardo Camacho Luis

*Por su participación como Asistente en las Jornadas Académicas "**La Investigación Científica, Compromiso y Pertinencia Social**", realizado los días 3, 4 y 5 de octubre del presente año en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED. La presente ampara 20 hrs. de actividad curricular.*

Atentamente

" Por mi raza hablará el espíritu "

Victoria de Durango, Dgo. a 05 de Octubre de 2018

Dr. Luis Francisco Sánchez Anguiano
Director del IIC

Dra. Laura Ernestina Barragán Ledesma
Representante de la DES
Ciencias de la Salud

UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO
FACULTAD DE AGRICULTURA Y ZOOTECNIA
VENECIA, DURANGO, MÉXICO

OTORGA LA PRESENTE

CONSTANCIA

A: ABELARDO CAMACHO LUIS

Maricela Esteban Méndez, Sandra C. Chávez Ramírez

Por su participación en la modalidad de cartel, con su trabajo de investigación:

FITOQUÍMICA Y EVALUACIÓN DE LA ACTIVIDAD ANTIBACTERIANA DE LA GUÁCIMA *Guazuma ulmifolia*

Presentado en la 29a. SEMANA INTERNACIONAL DE AGRONOMÍA
Celebrada del 04 al 08 de Septiembre del 2017 en el
Centro de Convenciones Posada del Río en Gómez Palacio, Dgo.

"POR MI RAZA HABLARÁ EL ESPÍRITU"
Venecia, Dgo. 08 de Septiembre del 2017

Ph. D. Juan José Martínez Ríos
Director

FACULTAD DE AGRICULTURA Y ZOOTECNIA
UJED
DIRECCIÓN

M.C. Diana Escobedo López
Secretaria Académica

El **Gobierno del Estado de Durango** a través de la **Secretaría de Salud** otorga la presente

Constancia

A:

Araujo Contreras Jesús María, Vargas Chávez Nohé, Rivas Ávila Efrén, Ávila Rodríguez Armando, Ávila Rodríguez E. Humberto, Camacho Luis Abelardo Reyes Romero Miguel Arturo.

Por su participación con el trabajo:

CORRELACIÓN ENTRE LOS ÍNDICES DE ADIPOSIDAD Y EL PORCENTAJE DE GRASA CORPORAL EN ADULTOS DE DURANGO.

en el marco de la I Jornada Nacional de Investigación en Salud Durango 2017

José Rosas Aispuro Torres
Gobernador del Estado de Durango

Dr. César Humberto Franco Mariscal
Secretario de Salud y Dir. Gral. de los Servicios de Salud

Victoria de Durango, Dgo. a Agosto de 2017

SLAN
Sociedad Latinoamericana de Nutrición

XVIII Congreso Latinoamericano de Nutrición
ALIMENTACIÓN SALUDABLE PARA UN PLANETA SOSTENIBLE
DEL 11 AL 15 DE NOVIEMBRE, 2018 » GUADALAJARA, JALISCO, MÉXICO

Otorga la presente

Constancia

a

ABELARDO CAMACHO LUIS

Por haber participado como PONENTE con el tema **"Relación entre grasa corporal y resistencia a la insulina en mujeres de Durango-Mx"** dentro de la sesión de carteles del **XVIII Congreso Latinoamericano de Nutrición** celebrado en Guadalajara, Jalisco, México, del 11 al 15 de noviembre de 2018.

AUTORES:
ARMANDO AVILA RODRÍGUEZ, ABELARDO CAMACHO LUIS, JOSÉ ALBERTO PÉREZ DE LA CRUZ, NOHÉ VARGAS CHÁVEZ, JESÚS MARÍA ARAUJO CONTRERAS, EFRÉN RIVAS AVILA, MIGUEL ARTURO REYES ROMERO

Dr. Juan Ángel Rivera Dommarco
Presidente de la Sociedad Latinoamericana de Nutrición

SLAN
2015 • 2018

El **Gobierno del Estado de Durango** a través de la
Secretaría de Salud otorga la presente

Constancia

A:

CAMACHO LUIS ABELARDO

Por su asistencia a la I Jornada Nacional de Investigación en Salud Durango 2017
Los días 30, 31 de Agosto y 1 de septiembre de 2017
Duración 16 horas con valor curricular

José Rosas Aispuro Torres
Gobernador del Estado de Durango

Dr. César Humberto Franco Mariscal
Secretario de Salud y Dir. Gral. de los Servicios de Salud

Victoria de Durango, Dgo. a Agosto de 2017

Objetivo: Contribuir a la difusión de los resultados de la investigación en el área de salud, así como promover el fortalecimiento del desarrollo de la investigación científica nacional.

Dirección de Enseñanza, Capacitación, Calidad e Investigación en Salud

	HORAS	CREDITOS
TEORIA	16	2
PRACTICA	0	0
TOTAL	16	2

Folio: 3925

SECRETARIA DE SALUD
SERVICIOS DE SALUD DE DURANGO

Coordinación Estatal de Capacitación

	DESCRIPCIÓN	HORAS
1	Diagnóstico molecular: Biología molecular aplicada en la práctica clínica.	1
2	Gestión de recursos de financiamiento para la investigación	1
3	Tomando mejores decisiones clínicas basadas en evidencia científica	1
4	Consentimiento informado en la práctica clínica y la investigación	1
5	Investigación y Desarrollo de Medicamento"	1
6	Alcances de la Inmunogenética en México y su aplicación en Trasplantes de Células Progenitoras Hematopoyéticas: Registro Mexicano de Donadores Altruistas de Médula Ósea"	1
7	Exposición de trabajos de investigación en las modalidades de Poster Científico y Ponencias orales del XV Concurso de investigación.	10
	Horas crédito	16

UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO

INSTITUTO DE INVESTIGACIÓN CIENTÍFICA

Otorga la presente:

Constancia

A

Esteban- Méndez M, Calzada – Contreras P.L., Camacho- Luis A., Ávila-Rodríguez A.

Por la presentación del trabajo ***"CARACTERIZACIÓN DE BACTERIAS ÁCIDO LÁCTICAS EN QUESO MENONITA PRODUCIDO EN NUEVO IDEAL DURANGO",*** *realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

Atentamente

" Por mi raza hablará el espíritu "

Victoria de Durango, Dgo. a 05 de Octubre de 2018

Dr. Luis Francisco Sánchez Anguiano
Director del IIC

Dra. Laura Ernestina Barragán Ledesma
Representante de la DES
Ciencias de la Salud

El **Gobierno del Estado de Durango** a través de la **Secretaría de Salud** otorga la presente

Constancia

A:

Vargas Chávez Nohé, Araujo Contreras Jesús María, Rivas Ávila Efrén, Ávila Rodríguez Armando, Ávila Rodríguez E. Humberto, Camacho Luis Abelardo y Reyes Romero Miguel Arturo.

Por su participación con el trabajo:

INDICADORES DE ADIPOSIDAD COMO FACTORES DE RIESGO DE INSULINORESISTENCIA EN ADULTOS DE LA CIUDAD DE DURANGO.

en el marco de la I Jornada Nacional de Investigación en Salud Durango 2017

José Rosas Aispuro Torres
Gobernador del Estado de Durango

Dr. César Humberto Franco Mariscal
Secretario de Salud y Dir. Gral. de los Servicios de Salud

Victoria de Durango, Dgo. a Agosto de 2017

El **Gobierno del Estado de Durango** a través de la **Secretaría de Salud** otorga la presente

Constancia

A:

Camacho Luis A. , Ávila Rodríguez A. , Victorica Galaviz B. , Loera Castañeda V. , Esteban Méndez M.

Por su participación con el trabajo:

Asociación de marcadores bioquímicos con envejecimiento exitoso.

en el marco de la I Jornada Nacional de Investigación en Salud Durango 2017

José Rosas Aispuro Torres

Gobernador del Estado de Durango

Dr. César Humberto Franco Mariscal

Secretario de Salud y Dir. Gral. de los Servicios de Salud

Victoria de Durango, Dgo. a Agosto de 2017

El **Gobierno del Estado de Durango** a través de la **Secretaría de Salud** otorga la presente

Constancia

A:

Liliana Guadalupe García Lara, Daniel Armando Carrillo García, Alejandra Guadalupe Gurrola Rodríguez, Karen del Carmen Lozoya Hernández, Diana Carcaño Zamora, Jennifer Jazmín Reyes Moreno, Elba Paloma Martínez Guillen, Abelardo Quiñones Márquez, Abelardo Camacho Luis, Méndez Hernández Edna Madai

Por su participación con el trabajo:

Eficacia del software educativo "Aula Interactiva De Nutrición" para mejorar el nivel de conocimiento nutricional en niños en edad preescolar.

En el marco de la I Jornada Nacional de Investigación en Salud Durango 2017

José Rosas Aispuro Torres
Gobernador del Estado de Durango

Dr. César Humberto Franco Mariscal
Secretario de Salud y Dir. Gral. de los Servicios de Salud

Victoria de Durango, Dgo. a Agosto de 2017

UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO
FACULTAD DE AGRICULTURA Y ZOOTECNIA
VENECIA, DURANGO, MÉXICO

OTORGA LA PRESENTE

CONSTANCIA

A: MARICELA ESTEBAN MÉNDEZ

Camacho Luis Abelardo, González Castillo María P.

Por su participación en la modalidad de cartel, con su trabajo de investigación:

IMPORTANCIA DE LA DETERMINACIÓN DE ENTEROCOCOS EN QUESO DE BOVINO

Presentado en la 29a. SEMANA INTERNACIONAL DE AGRONOMÍA
Celebrada del 04 al 08 de Septiembre del 2017 en el
Centro de Convenciones Posada del Río en Gómez Palacio, Dgo.

"POR MI RAZA HABLARÁ EL ESPÍRITU"
Venecia, Dgo. 08 de Septiembre del 2017

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