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Research Article

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Facile Synthesis of Two Azete-Steroid Derivatives and Theoretical Evaluation of Its Interaction with the Aromatase Enzyme

İki Azete Steroid Türevinin Basit Sentezi ve Aromataz Enzimi ile Etkileşiminin Teorik Olarak Değerlendirilmesi

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ABSTRACT

Several aromatase inhibitors have used for the treatment of breast cancer; however, some of these drugs may produce some side effects such as endometrial cancer and bone loss. The aim of this study was to synthesize two new azete-steroid derivatives (compounds 9 or 10) to evaluate its theoretical interaction with an aromatase enzyme (2wd3) using anastrozole and exemestane as controls in a docking model. The preparation of 9 and 10 was carried out using a series of reactions which involves amination, etherification, nitration, and addition. Chemical structure of the compounds was confirmed using elemental analysis and NMR spectrum. The results showed that compounds 9 or 10 could bind to a different type of aminoacid residues involved in of 2wd3 protein surface compared anastrozole and exemestane; this phenomenon may exert changes in the biological activity of aromatase enzyme. All data suggest that compounds 9 or 10 could be an alternative for the treatment of breast cancer; therefore it could be a good candidate for the pharmaceutical industry.

Key Words

Azete, steroid, derivative, docking model.

ÖZ

Meme kanserinin tedavisi için birkaç aromataz inhibitörü kullanılmıştır; ancak, bu ilaçların bazıları endometrial kanser ve kemik kaybı gibi bazı yan etkiler yaratabilir. Bu çalışmanın amacı, bir yerleştirme modelinde kontroller olarak anastrozol ve exemestan kullanarak teorik etkileşimini bir aromataz enzimi (2wd3) ile teorik etkileşimini değerlendirmek için iki yeni azete-steroid türevini (bileşik 9 veya 10) sentezlemekti. 9 ve 10'un hazırlanması, aminasyon, eterleştirme, nitrasyon ve ekleme içeren bir dizi reaksiyon kullanılarak gerçekleştirildi. Bileşiklerin kimyasal yapısı element analizi ve NMR spektrumu kullanılarak doğrulandı. Sonuçlar, bileşikler 9 veya 10'un, anastrozol ve exemestan ile karşılaştırıldığında 2wd3 protein yüzeyinde yer alan farklı tipte bir amino asit tortusuna bağlanabileceğini gösterdi; bu fenomen, aromataz enziminin biyolojik aktivitesinde değişiklikler yapabilir. Tüm veriler, bileşik 9 veya 10'un, meme kanseri tedavisinde bir alternatif olabileceğini göstermektedir; bu nedenle ilaç endüstrisi için iyi bir aday olabilir.

Anahtar Kelimeler

Azete, steroid, türev, yerleştirme modeli.

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INTRODUCTION

There are studies which indicate that breast cancer is one of the main health problems worldwide [1-4]. It is noteworthy that some drugs have been used to treatment of breast cancer such as tamoxifen (estrogen-receptor antagonist) [5], anastrozole, letrozole or exemestane (aromatase inhibitors) [6-8], fisetin or methyl paraben (17-hydroxy dehydrogenase type 1 inhibitors) [9, 10]; however, some of these drugs may produce some adverse effects such as secondary endometrial cancer [11] and bone loss [12]. In the search of new pharmacological treatment to breast cancer, some drugs have been developed; for example, the asymmetric synthesis of a piperidine derivative via an organocatalytic Michael-Henry reaction with biological activity against breast cancer in vitro [13]. In addition, a study showed the preparation of nimesulide from 2-Amino-5-nitro-phenol as a breast cancer inhibitor using SK-BR-3 cells [14]. Also, a series of dienone-derivatives were synthesized from oridonin which exerted effects against breast cancer in vitro [15]. Other study showed reaction of 4-(dimeth-ylamino)benzaldehyde with N-(3-Acetyl-2-hydroxy-phenyl)-acetamide to form 8-Amino-4′-(dimethylamino)flavone and their biological activity against breast cancer on MCF-7 cells [16]. It is important to mention, that some theoretical models have been used to characterize the interaction of drugs with some biomolecules involved in breast cancer; for example, a theoretical analysis showed the interaction plumbagin-hydrazine (breast cancer inhibitor) with NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) using a Docking model [17], Additionally, other report showed the interaction of tamoxifen, raloxifene, toremifene (breast cancer inhibitors) with estrogen receptor using the HEX-docking software [18]. Other data showed that some estrone derivatives can produce apoptosis of breast cancer cells via carbonic anhydrase IX inhibition using Autodock program [19]. All these data indicate that several drugs could exert effects on breast cancer; however, their interaction with several targets biological is confusing, this phenomenon could be due to the different chemical structures of each drug or to protocols used. Analyzing these data, the aim of this study was to synthetize two azete-steroid derivatives were prepared and their theoretical activity on aromatase enzyme was evaluated using a Docking-Server model.

MATERIALS and METHODS

2.1 General methods

The reagents involved in this investigation were purchased from Sigma-Aldrich Sigma-Aldrich Co., Ltd. The melting point for compounds was determinate using an Electrothermal (900 model). Infrared spectra (IR) were evaluated using i50 FT-IR Nicolet spectrometer.^{1}H and ^{13}C NMR (nuclear magnetic resonance) spectra were determinate with a Varian VXR300/5 FT NMR spectrometer at 300 MHz (megahertz) in $CDCl_3$ (deuterated chloroform). EIMS (electron impact mass spectroscopy) spectra were evaluated using a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were evaluated from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

Chemical Synthesis

Amination

In a round bottom flask (10 ml), 2-nitroestrone or 2-nitroestradiol (0.50 mmol), 3-Ethynylanyline (60 µl 0.53 mmol) and 5 ml of formaldehyde were stirred to reflux for 12h. The mixture was purified via a crystallization using the methanol:water (4:1) system.

4-[(3-Ethynyl-phenylamino)-methyl]-3-hydroxy-13-methyl-2-nitro-6,7,8,9,11,12,13,14,15,16-decahydro-cyclopenta[a]phenanthren-17-one (3)

yielding 44% of product; m.p. 43-45°C; IR (vmax, cm^{-1}) 3402, 3312, 2102, 1712 and 1352: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.93 (s, 3H), 1.20-1.90 (m, 7H), 2.12-2.50 (7H), 2.82 (s, 1H), 3.01 (m, 1H), 4.40 (m, 2H), 6.56-7.12 (m, 4H), 7.16 (m, 1H), 9.10 (broad, 2H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 13.9, 21.6, 25.4, 27.4, 28.0, 30.8, 34.6, 37.2, 42.3, 47.9, 48.4, 50.4, 78.2, 84.0, 113.1, 122.5, 122.7, 123.5, 125.0, 125.0, 129.8, 134.3, 136.6, 145.4, 146.7, 147.1, 219.8 ppm. EI-MS m/z: 444.53. Anal. Calcd. for $C_{27}H_{28}N_2O_4$: C, 72.95; H, 6.35; N, 6.30; O, 14.40. Found: C, 72.90; H, 6.28.

4-[(3-Ethynyl-phenylamino)-methyl]-13-me- thyl-2-nitro-7,8,9,11,12,13,14,15,16,17-deca- hydro-6H-cyclopenta[a]phenanthrene-3,17-diol (4)

yielding 56% of product; m.p. 58-60°C; IR (vmax, cm^{-1}) 3400, 3310, 2102 and 1350: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.64 (s, 3H), 0.80-1.86 (m, 11H), 2.12-2.52 (m, 3H), 2.84 (s, 1H), 2.94-3.64 (m, 2H), 4.40 (m, 2H), 6.62-7.10 (m, 4H), 7.64 (m, 1H), 8.20 (broad, 3H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 15.8, 24.2, 25.3, 27.7, 28.0,

32.7, 33.7, 37.2, 42.3, 44.4, 45.1, 50.7, 78.2, 82.4, 84.01, 113.1, 122.4, 122.7, 123.5, 125.0, 125.3, 129.8, 134.8, 136.6, 145.4, 147.0, 147.1 ppm. EI-MS m/z: 446.22. Anal. Calcd. for $C_{27}H_{30}N_2O_4$: C, 72.62; H, 6.77; N, 6.27; O, 14.33. Found: C, 72.58; H, 6.70.

Etherification

In a round bottom flask (10ml), compounds 3 or 4 (0.50 mmol), potassium carbonate (60 mg, 0.43) 5 ml of dimethyl sulfoxide were stirred to reflux for 6h. The solvent of mixture was reduced pressure and purified via a crystallization using the methanol:water (4:1) system.

4-[(3-Ethynyl-phenylamino)-methyl]-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-20-oxa-cyclopropa[2,3]cyclopenta[a]phenan- thren-17-one (5)

yielding 38% of product; m.p. 76-78°C; IR (vmax, cm^{-1}) 3310, 2104, 1712 and 1242: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.90 (s, 3H), 1.20-1.92 (m, 7H), 2.12-2.54 (m, 8H), 2.88 (s, 1H), 4.48 (m, 2H), 4.73 (broad, 1H), 6.22 (m, 1H), 6.62-7.16 (m, 4H) ppm. 13.8, 21.7, 25.7, 27.4, 28.0, 31.3, 35.1, 37.5, 38.3, 47.4, 48.2, 50.5, 78.2, 84.0, 109.8, 113.1, 118.3, 122.5, 122.7, 125.0, 129.8, 131.9, 133.1, 143.6, 147.4, 148.5, 220.7 ppm. EI-MS m/z: 397.20. Anal. Calcd. for $C_{27}H_{27}NO_2$: C, 81.58; H, 6.85; N, 3.52; O, 8.05. Found: C, 81.50; H, 6.80.

4-[(3-Ethynyl-phenylamino)-methyl]-13-methyl-6,8,9,11,12,13,14,15,16,17-decahydro-7H-20-oxa-cyclopropa[2,3]cyclopenta[a]phe- nanthren-17-ol (6)

yielding 45% of product; m.p. 84-86°C; IR (vmax, cm^{-1}) 3400, 3312, 2102 and 1240: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.76 (s, 3H), 0.82-1.88 (m, 11H), 2.12-2.54 (m, 4H), 2.88 (s, 1H), 3.64 (m, 1H), 4.48 (m, 2H), 5.58 (broad, 2H), 6.16 (m, 1H), 6.62-7.16 (m, 4H) ppm. 15.8, 24.2, 25.3, 27.7, 28.0, 32.7, 33.7, 37.2, 38.3, 44.4, 44.6, 50.7, 78.2, 82.4, 84.0, 110.2, 113.1, 118.3, 122.5, 122.7, 125.0, 129.8, 132.3, 133.5, 143.6, 147.4, 148.5 ppm. EI-MS m/z: 399.21. Anal. Calcd. for $C_{27}H_{29}NO_2$: C, 81.17; H, 7.32; N, 3.51; O, 8.01. Found: C, 81.12; H, 7.30.

Nitration

In a round bottom flask (10ml), compounds 5 or 6 (0.50 mmol), 5 ml of anhydride acetic and 1 ml of nitric acid were stirred to reflux for 6h. The solvent of mixture was reduced pressure and purified via a crystallization using the methanol:hexane:water (4:2:1) system.

4-[(3-Ethynyl-2,4,5,6-tetranitro-phenylamino)-methyl]-13-methyl-1-nitro-6,7,8,9,11, 12,13,14,15,16-decahydro-20-oxa-cyclopro- pa[2,3] cyclopenta[a]phenanthren-17-one (7)

yielding 66% of product; m.p. 122-124°C; IR (vmax, cm^{-1}) 3312, 1244, 1712 and 1352: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.92 (s, 3H), 1.20-1.91 (m, 7H), 2.12-3.00. (m, 15H), 3.90 (s, 1H), 4.84 (m, 2H), 9.92 (broad, 1H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 13.8. 21.7, 27.4, 28.1, 30.0, 31.3, 35.3, 37.7, 42.3, 45.2, 48.1, 50.2, 71.0, 79.7, 115.2, 125.1, 126.2, 128.5, 130.2, 132.5, 134.4, 135.1, 136.3, 136.5, 141.9, 142.3, 220.3 ppm. EI-MS m/z: 6.22.12. Anal. Calcd. for $C_{27}H_{22}N_6O_{12}$: C, 52.09; H, 3.56; N, 13.50; O, 30.84. Found: C, 52.00; H, 3.50.

4-[(3-Ethynyl-2,4,5,6-tetranitro-phenylamino)-methyl]-13-methyl-1-nitro-6,8,9,11,12, 13,14,15,16,17-decahydro-7H-20-oxa-cyclopropa[2,3]cyclopenta[a]phenanthren-17-ol (8)

yielding 58% of product; m.p. 135-137°C; IR (vmax, cm^{-1}) 3400, 3312, 2102, 1350 and 1242: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.78 (s, 3H), 0.80-1.86 (m, 9H), 2.22-3.64 (m, 7H), 3.92 (s, 1H), 4.82 (m, 2H), 8.12 (broad, 2H), 6.62-7.12 (m, 4H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$) δ_C: 15.8, 24.2, 27.6, 27.7, 30.0, 32.8, 33.7, 37.6, 42.3, 44.3, 50.7, 71.0, 79.70, 82.4, 115.2, 125.1, 126.2, 129.0, 130.6, 132.5, 134.8, 135.12, 136.3, 136.5, 141.9, 142.3 ppm. EI-MS m/z: 6.24.14. Anal. Calcd. for $C_{27}H_{24}N_6O_{12}$: C, 51.93; H, 3.87; N, 13.46; O, 30.74. Found: C, 51.88; H, 3.80.

Synthesis of azete derivatives

In a round bottom flask (10ml), compounds 7 or 8 (0.50 mmol), 4-Nitrophenylacetonitrile (85 mg, 0.52 mmol), CopperII chloride anhydrous (67 mg, 0.50 mmol) and 5 ml of methanol were stirred to room temperature for 48h. The solvent of mixture was reduced pressure and purified via a crystallization using the methanol:hexane:water (4:2:1) system;

13-Methyl-1-nitro-4-({2,3,4,6-tetranitro-5-[2-(4-nitro-benzyl)-azet-3-yl]-phenylamino}-methyl)-6,7,8,9,11,12,13,14,15,16-decahydro-20-oxa-cyclopropa[2,3]cyclopenta[a] phenanthren-17-one (9)

yielding 72% of product; m.p. 156-158°C; IR (vmax, cm^{-1}) 3312, 1712, 1350 and 1154: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.92 (s, 3H), 1.20-1.91 (m, 7H), 2.12-3.00 (m, 8H), 3.77 (m, 2H), 4.82 (broad, 1H), 5.70 (s, 1H),

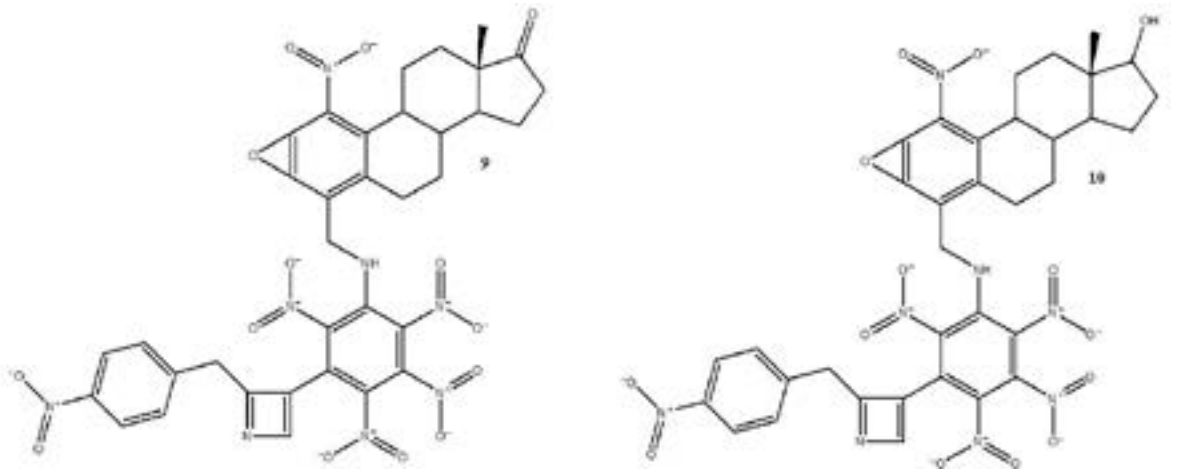

Figure 1. Chemical structure of two azete-steroid derivatives (9 and 10).

7.33-7.92 (m 4H), 9.32 (broad, 1H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$)δ_C: 13.8. 21.7, 27.4, 28.1, 30.0, 31.3, 35.3, 37.7, 42.3, 44.5, 45.2, 48.1, 50.2, 78.2, 112.6, 114.4, 117.8, 124.1, 124.6, 125.7, 126.0, 128.1, 128.6, 130.2, 130.3, 133.7, 134.4, 135.7, 135.8, 136.3, 137.4, 138.3, 142.0, 146.6, 179.2, 220.3 ppm. EI-MS m/z: 784.17. Anal. Calcd. for $C_{35}H_{28}N_8O_{14}$: C, 53.58; H, 3.60; N, 14.28; O, 28.55. Found: C, 53.50; H, 3.54.

13-Methyl-1-nitro-4-({2,3,4,6-tetranitro-5-[2-(4-nitro-benzyl)-azet-3-yl]-phenylamino}-methyl)-6,8,9,11,12,13,14,15,16,17-decahy- dro-7H-20-oxa-cyclopropa[2,3]cyclopenta [a]phenanthren-17-ol (10) yielding 68% of product; m.p. 172-174°C; IR (vmax, cm^{-1}) 3400, 3312, 1352 and 1154: ^{1}H NMR (300 MHz, Chloroform-d) δ_H: 0.78 (s, 3H), 0.80-1.86 (m, 9H), 2.20-3.62 (m, 7H), 3.77 (m, 2H), 4.82 (broad, 1H), 5.70 (s, 1H), 7.33 (m, 2H), 7.86 (broad, 2H), 7.92 (m 2H) ppm. ^{13}C NMR (300 Hz, $CDCl_3$)δ_C: 15.8, 24.2, 27.7, 30.0, 32.7, 33.7, 37.7, 42.3, 42.3, 44.4, 44.5, 50.7, 82.4, 117.8, 124.1, 124.6, 125.7, 126.0, 128.1, 128.9, 130.2, 130.6, 133.7, 134.8, 135.7, 135.8, 136.3, 137.4, 138.3, 142.0, 146.6, 179.2 ppm. EI-MS m/z: 786.18. Anal. Calcd. for $C_{35}H_{30}N_8O_{14}$: C, 53.44; H, 3.84; N, 14.24; O, 28.47. Found: C, 53.38; H, 3.80.

2.3 Physicochemical parameters evaluation

Some electronic parameters such as HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital) energy, orbital coefficients distribution, molecular dipole moment and HBD (hydrogen bond donor groups) and HBA (hydrogen bond acceptor groups) and PSA (polar surface area) were evaluated using the SPARTAN'06 software [20].

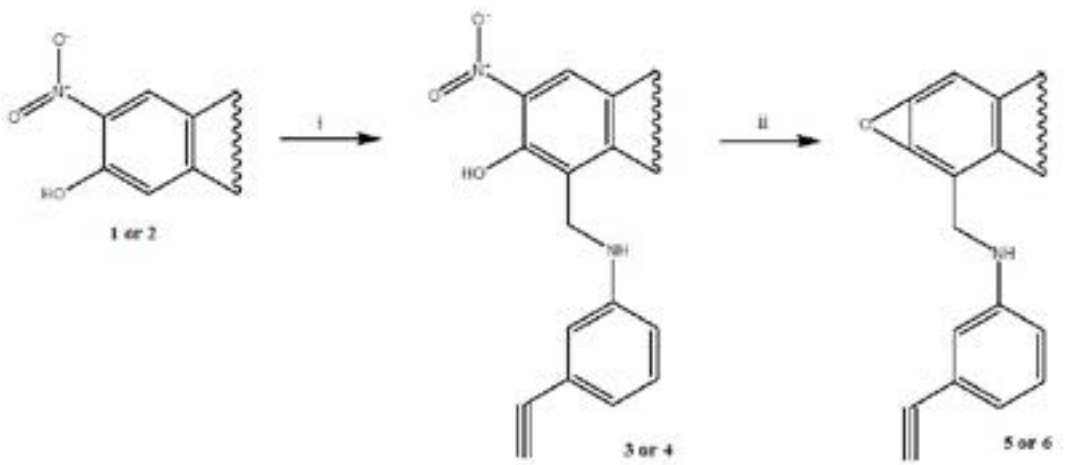

Figure 2. Synthesis of two ether-steroid derivatives (5 or 6). Reaction of 2-Nitroestrone (1) or 2-Nitroestradiol (2) with 3-Ethynylanyline (i) to form 4-[(3-Ethynyl-phenylamino)-steroid-17-one (3) or 4-[(3-Ethynyl-phenylamino)-steroid-3,17-diol (4). Then, the compound 5 or 6 were prepared via intramolecular displazament of nitrile group involved in the chemical structure from 3 or 4. ii = dimethyl sulfoxide.

Pharmacophore evaluation

The 3D pharmacophore model for the compounds 6 and 7 was determinate using LigandScout 4.08 software [21]

2.5 Theoretical evaluation of the interaction between compounds 9 or 10 and aromatase enzyme.

The interaction of compound 4 with aromatase enzyme (2wd3) [22] was carried out using a DockingServer [23].

RESULTS and DISCUSSION

In this study were prepared two azete-steroid derivatives (Figure 1) from 2-nitroestrone (compound 1) or 2-nitroestradiol (compound 2) using some chemical strategies.

The first stage was achieved for the synthesis of three amino steroids; it is important to mention that several methods have been used to synthesis some amino-steroid derivatives for example, the preparation of 17-α-amino steroids using a ω-transaminase enzyme from Arthrobacter sp [23]. Other studies showed the synthesis of amino-steroids through a nitro-steroids derivatives reduction [24]. Also, a report showed an amination of steroids using palladium as catalyst [25]. Other data showed the synthesis of some amino-steroid derivatives via Mannich reaction; it is noteworthy that structural chemistry of these compounds [26] involves an activated methyl group in ring A.

In this study, the reactivity of the hydrogen atom involved in ring A (C-4) of both compounds 1 or 2 was evaluated using the Mannich reaction. Therefore, 1 or 2 reacted with 3-ethynyl-aniline in presence of formaldehyde (Figure 2) to form the amino-steroid derivatives (compounds 3 and 4). The results of ^{1}H NMR spectrum for 3 showed several signals at 0.93 ppm for methyl group bound to steroid nucleus; at 1.20-2.50, 3.01 and 7.16 ppm for steroid moiety; at 2.82 ppm for alkyne group; at 4.40 for methylene bound to both amino group and ring A; at 6.56-7.12 ppm for phenyl group; at 9.10 ppm for both hydroxyl and amino groups. The ^{13}C NMR showed several signals at 13.9 ppm for methyl group bound to steroid nucleus; at 21.6-37.2, 47.9-50.4, 123.5-125.0 and 134.3-146.7 ppm for steroid moiety; at 42.34 ppm for methylene group bound to both amino group and ring A; at 78.2-84.0 ppm for alkyne group; at 113.1-122.7, 125.0-129.8 and 147.1 ppm for phenyl group; at 219.8 ppm for ketone group. Finally, the mass spectrum from 3 showed a molecular ion (m/z) 444.53.

Other results showed several signals involved in the ^{1}H NMR spectrum for 4 at 0.64 ppm for methyl group; at 0.80-2.52, 2.94-3.64 and 7.64 ppm for steroid moiety; at 2.84 ppm for alkyne group; at 4.40 ppm for methylene bound to amino group and ring A; at 6.62-7.10 for phenyl group; at 8.20 ppm for both hydroxyl and amino groups. The ^{13}C NMR showed several signals at 13.8 ppm for methyl bound to steroid nucleus; at 24.2-37.2, 44.2-50.7, 86.4, 123.5, 125.3, 134.8-145.4 and 147.1 ppm for steroid moiety; at 42.3 ppm for methylene bound to amino group and ring A; at 78.2-84.0 ppm for alkyne group; at 113.1-122.7, 125.00, 129.8 and 147.0 ppm for phenyl group; at 220.3 ppm for ketone group.

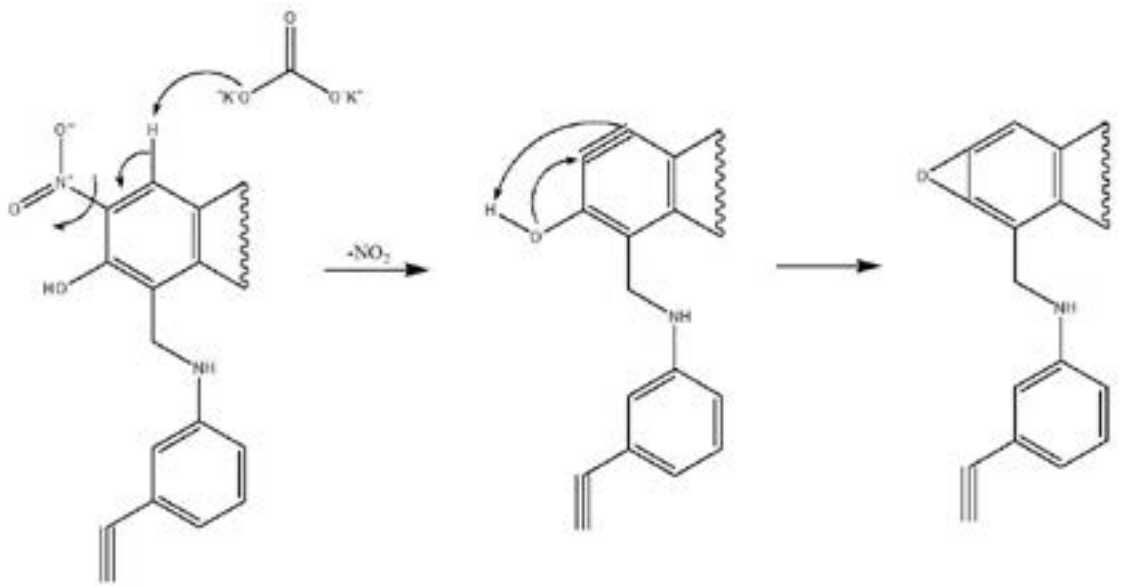

Figure 3. Reaction mechanism of synthesis of compounds 5 or 6.

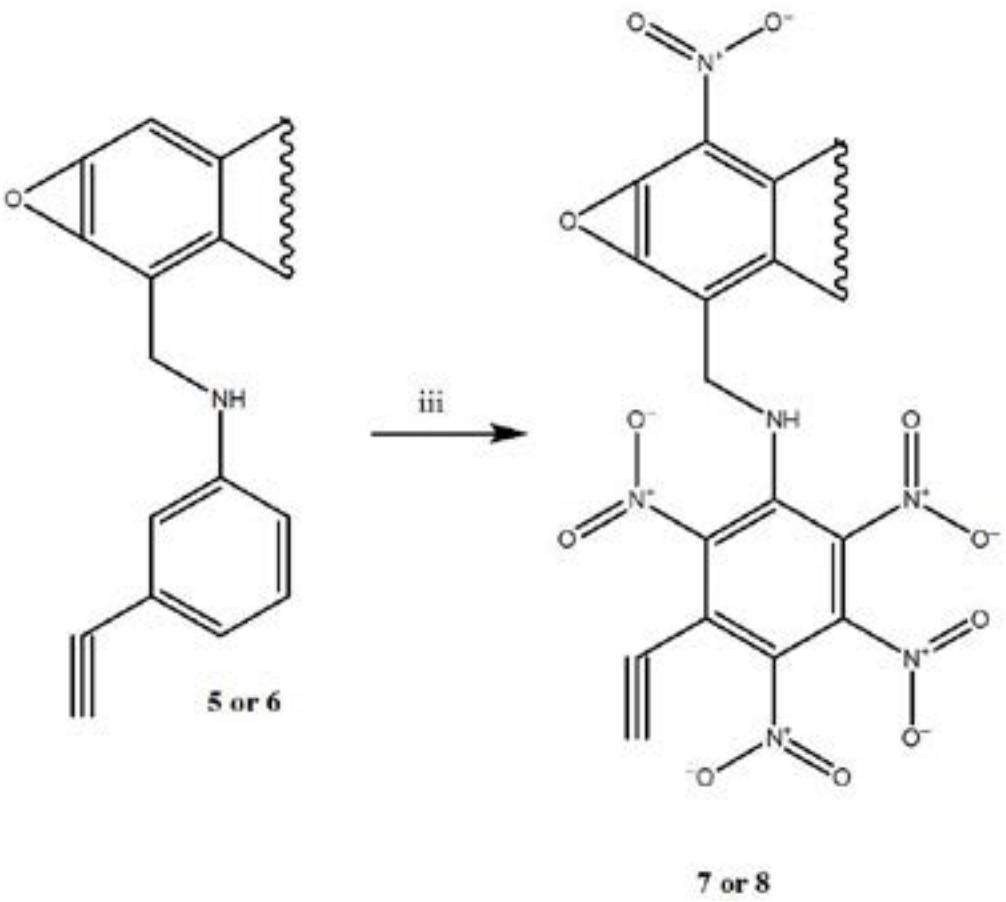

Figure 4. Synthesis of two tetranitro-phenylamino)-1-nitro-steroid derivatives (7 or 8). Nitration of two ether-steroid derivatives (5 or 6) with HNO3/(CH3CO)2O (iii) to form 7 or 8.

In addition, the mass spectrum from 4 showed a molecular ion (m/z) 446.22.

Etherification of 3 or 4

Several studies showed the preparation of ether groups using some reagents such as (E)-alk-2-en-ol [27], CuCl [28], $Cu(AcO)_2$ [29], PhCOOH [30]. In addition, there are some methods to the synthesis of ether groups, via displacement of nitro groups using dipolar aprotic solvents [31]. Analyzing these data, in this study, two ether-steroid derivatives (compound 5 or 6) were prepared using a previously method reported via intramolecular displacement of the nitro group from compounds 3 and 4 in the presence of dimethyl sulfoxide (Figure 2). In addition, the formation of 5 or 6 involves an cyclohexa-1,3-dien-5-yne as intermediary (Figure 3).

The results of ^{1}H NMR spectrum for 5 showed several signals at 0.90 ppm for methyl group bound to steroid nucleus; at 1.20-2.54 and 6.22 ppm for steroid moiety; at 4.48 ppm for methylene group bound to both amino and A-ring of steroid; at 2.88 ppm for alkyne group; at 4.73 ppm for amino group; at 6.62-7.16 ppm for phenyl group. The ^{13}C NMR displayed several signals at 13.8 ppm for methyl group bound to steroid nucleus; at 21.7-35.7, 47.4-50.5, 109.8, 118.3 and 131.9-147.4 ppm for steroid moiety; at 38.44 ppm for methylene bound to both amino and A-ring; at 78.2-84.0 ppm for alkyne group; at 113.1, 122.5-129.8 and 148.5 ppm for phenyl group; at 220.70 for ketone group. Finally, the mass spectrum from 5 showed a molecular ion (m/z) 397.20.

Other results showed several signals involved in the ^{1}H NMR spectrum for 6 at 0.76 ppm for methyl group bound to steroid nucleus; at 0.82-2.54, 3.64 and 6.16 ppm for steroid moiety; at 2.88 ppm for alkyne group; at 4.48 ppm for methylene bound to both amino and A-ring; at 5.58 ppm for both hydroxyl and amino groups; at 6.62-7.16 ppm for phenyl group. The ^{13}C NMR showed several signals at 15.82 ppm for methyl group bound to steroid nucleus; at

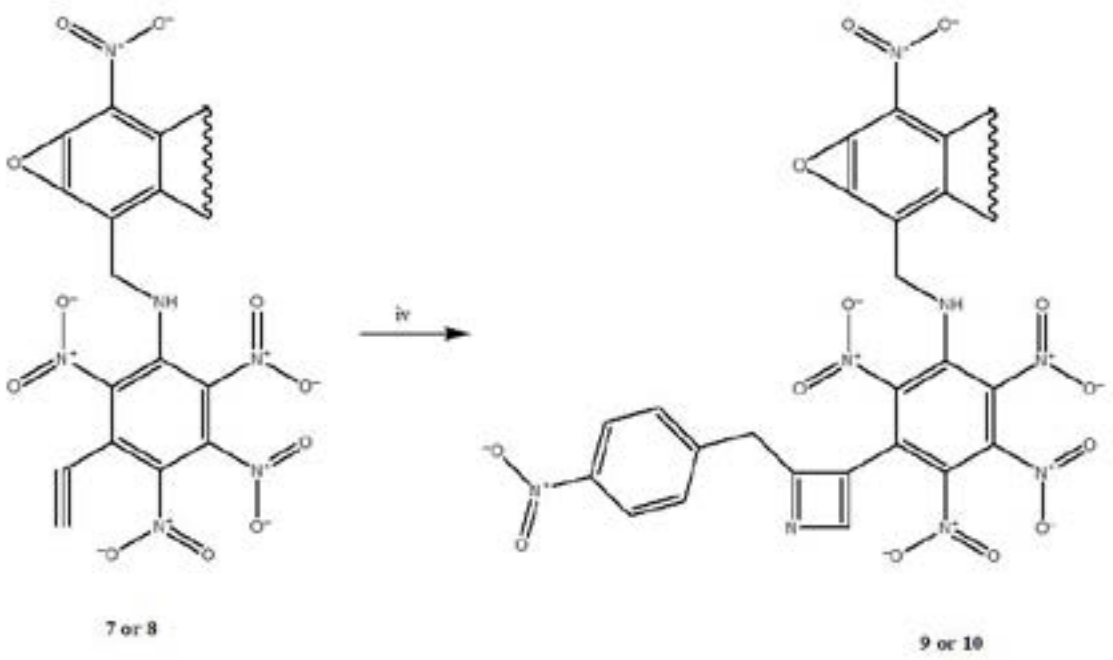

Figure 5. Preparation of two azete-steroid derivatives (9 or 10). Reaction of 7 or 8 with 4-Nitrophenylacetonitrile to form 9 or 10 using CopperII (iv) as catalyst.

24.2-37.2, 44.4-50.7, 82.4, 110.2, 118.3 and 132.3-147.4 ppm for steroid moiety; at 38.4 ppm for methylene bound to both amino group and A-ring; at 78.21 and 84.00 for alkyne group; at 113.14, 122.5-129.8 and 148.5 ppm for phenyl group. Additionally, the mass spectrum from 6 showed a molecular ion (m/z) 399.21.

Nitration of 5 or 6

There are several report in the literature for preparation of nitro derivatives usin g some reagents such as WO_3/ZrO_2 (32), NO_2 [33], HNO_3/montmorillonite [34], NH_4NO_3 and $(CF_3\ CO)_2O$ [35], BF_3 [36], HNO_3/H_2SO_4 [37] and others. In this study, compounds 7 or 8 (Figure 4) were prepared through of nitration of phenyl groups involved in the chemical structure of 5 or 6 with $HNO_3/(CH_3CO)_2O$. The ^{1}H NMR spectrum for 7 showed several at 0.92 ppm for methyl group bound to steroid nucleus; at 1.20-3.00 ppm for steroid moiety; at 3.90 ppm for alkyne group; at 4.84 ppm for methylene bound to both amino and phenyl groups; at 9.92 for amino group. The ^{13}C NMR displayed several signals at 13.8 ppm for methyl group bound to steroid nucleus; at 21.7-37.7, 45.2-50.2, 126.2-130.2, 134.4, 136.3 and 141.9 ppm for steroid moiety; at 42.3 ppm for methylene bound to both amino and phenyl groups; at 71.0-79.7 ppm for alkyne group; at 115.2-125.1, 132.5, 135.1, 136.5 and 142.3 ppm for phenyl group; at 220.30 ppm for ketone group. Finally, the mass spectrum from 7 showed a molecular ion (m/z) 6.22.12.

Other results showed several signal involved in the ^{1}H NMR spectrum for 8 at 0.78 ppm for methyl group bound to steroid nucleus; at 0.80-3.64 ppm for steroid moiety; at 3.92 ppm for alkyne group; at 4.82 ppm for methylene bound to both amino and phenyl groups; at 8.12 ppm for both hydroxyl and amino groups. The ^{13}C NMR showed several signals at 15.8 ppm for methyl group; at 24.2-37.6, 44.3-50.7, 82.4, 126.2-130.6, 134.8, 136.3 and 141.9 ppm for steroid moiety; at 42.3 ppm for methylene bound to both amino and phenyl groups; at 71.0-79.7 ppm for alkyne group; at 115.2-125.2, 132.5, 135.1, 136.5 and 142.3 ppm for phenyl groups. Additionally, the mass spectrum from 8 showed a molecular ion (m/z) 624.14.

Formation of Azete Derivatives (compound 9 or 10)

Several oxazete-derivatives have been synthetized using some reagents such as mesitonitrile oxide [38], α,α-bis-(alkylthio) oxime [39], acylisothiocyanate [40] and others. In this investigation, two azete derivatives were prepared via reaction 2 + 2 addition of compounds 7 or 8 and 1-Nitro-4-prop-2-ynyl-benzene using CopperII chloride as catalyst (Figures 5 and 6).

The ^{1}H NMR spectrum for 9 (Figure 7) at 0.92 ppm for methyl group bound to steroid nucleus; at 1.20-3.00 ppm for steroid moiety; at 3.77 for methylene bound to both phenyl group and azete ring; at 4.82 ppm for methylene

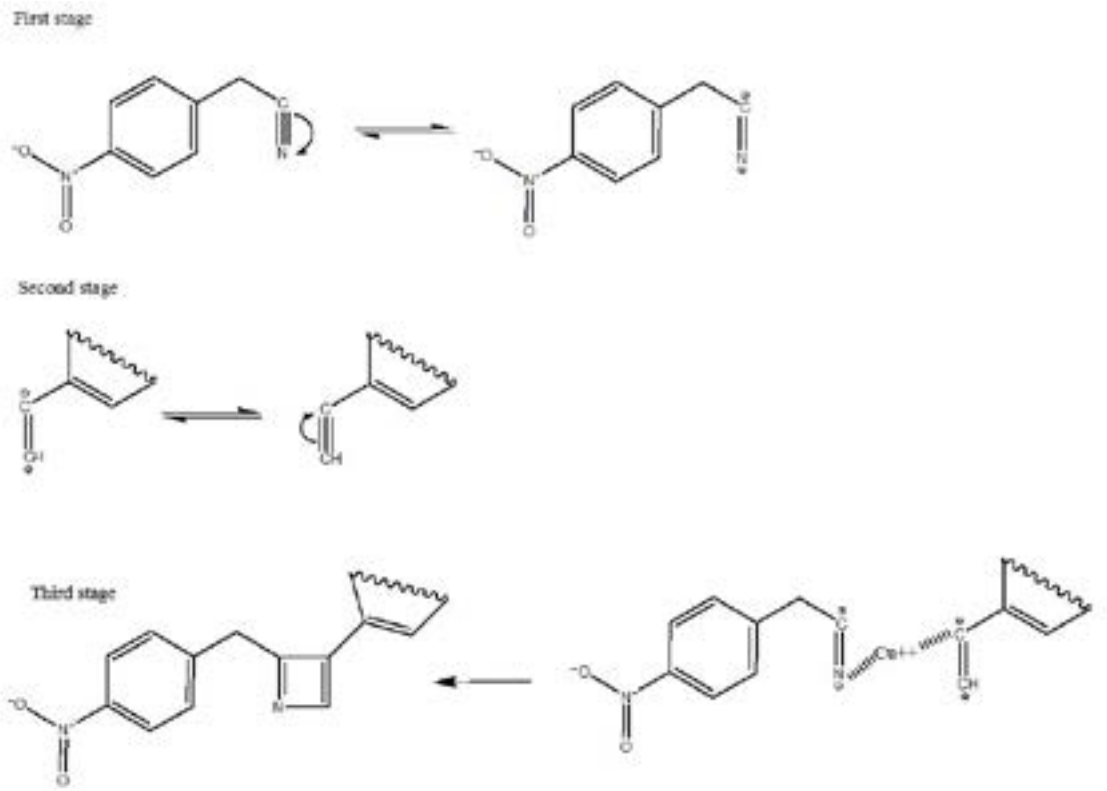

Figure 6. Reaction mechanism of synthesis of compounds 9 or 10.

bound to both amino and phenyl group; at 5.70 ppm for azete ring; at 7.33-7.92 ppm for phenyl group; at 9.32 for amino group.

The ^{13}C NMR displayed several signals at 13.8 ppm for methyl group; at 21.7-37.7, 45.2-50.2, 124.1, 128.6, 130.3, 134.4, 136.3 and 142.0 ppm for steroid moiety; at 42.3 ppm for methylene bound to both phenyl and amino groups; at 44.5 ppm for methylene bound to phenyl group and azete ring; at 117.8, 138.3 and 179.2 ppm for azete ring; at 124.6-128.1, 130.2, 133.7, 135.7-135.8, 137.4 and 146.6 ppm for phenyl groups; at 220.30 for ketone group. Additionally, the mass spectrum from 9 showed a molecular ion (m/z) 784.17.

Finally, the ^{1}H NMR spectrum for 10 (Figure 8) showed several signals at 0.78 ppm for methyl group bound to steroid nucleus; at 0.80-3.62 ppm for steroid moiety; at 3.77 ppm for methylene bound to both phenyl group and azete ring; at 4.82 ppm for methylene bound to both amino and phenyl groups; at 5.70 ppm for azete ring; at 7.36 and 7.92 ppm for phenyl group; at 7.86 ppm for both hydroxyl and amino groups.

The ^{13}C NMR showed several signals at 15.8 ppm for methyl group; at 24.2-37.7, 42.3-44.4, 50.7-82.4, 128.9, 130.6, 134.8, 136.3 and 142.0 ppm for steroid moiety; at 42.3 ppm for methylene bound to both amino and phenyl groups; at 44.5 ppm for methylene bound to both phenyl group and azete ring; at 117.8, 138.32 and 179.2 ppm for azete ring; at 124.1-128.1, 130.2, 133.7, 135.8, 137.4 and 146.6 ppm for phenyl groups. Additionally, the mass spectrum from 10 showed a molecular ion (m/z) 786.18.

Electronic Parameters

There are some studies which indicate that molecular orbitals and frontier electron density are used to predict the most reactive position in some electron system on several types of reactions [41, 42].These studies suggest that values of highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) and their energy gap reflect the chemical activity of a molecule [43].

Here, it is important to mention, that, some methods have been developed to evaluate the relation between HOMO and LUMO with biological activity of some

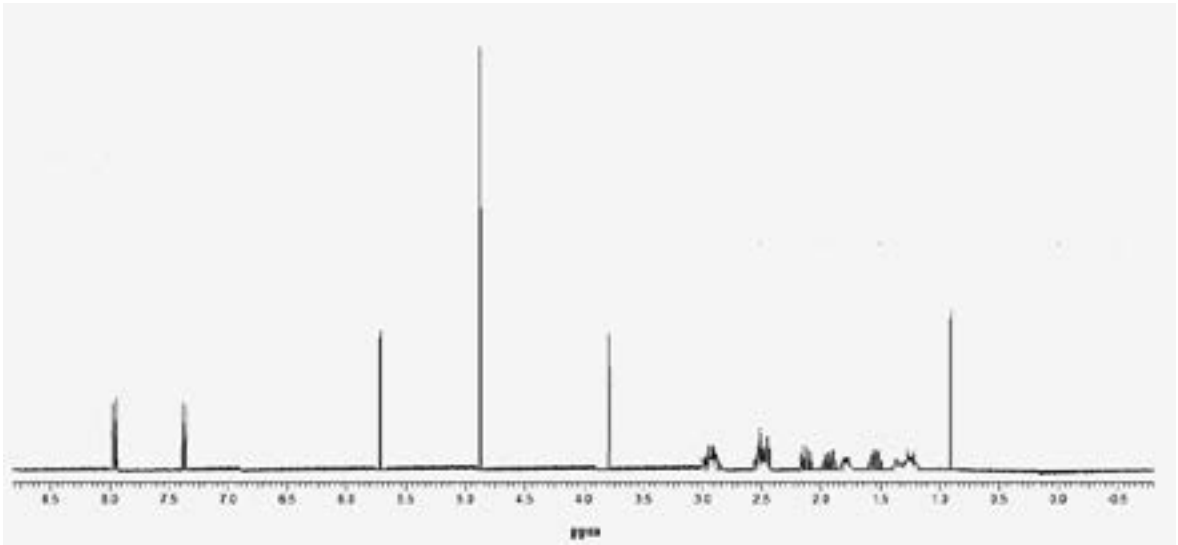

Figure 7. The scheme showed [1]HNMR spectrum from compound 9. The spectrum was analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in CDCl3. Axis abscissa (ppm). ppm = parts per million.

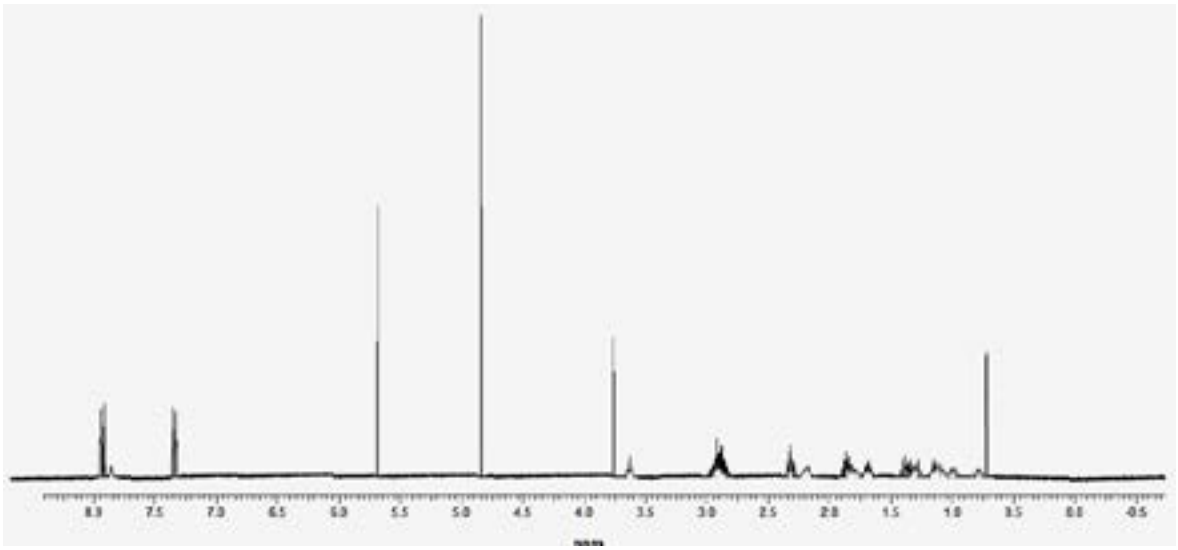

Figure 8. The scheme showed [1]HNMR spectrum from compound 10. The spectrum was analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. Axis abscissa (ppm). ppm = parts per million.

Table 1. Physicochemical parameters involve in the structure of compounds 9 and 10.

Parameters	C-9	C-10
Polarizability (cm^3)	93.12	93.52
PSA Å2	240.23	244.82
LogP	-1.38	-1.87
Energy (au)	-2605.54	-2606.72
HBD	1	2
HBA	19	19
HOMO (eV)	-8.46	-8.49
LUMO (eV)	-1.68	-1.66

HBD (hydrogen bond donors); HBA (hydrogen bond acceptors); PSA (polar surface area).

compounds; for example, there are some data which showed the evaluation of the frontier molecular orbitals (HOMO-LUMO gap) from some steroid using MINDO and ZINDO models [44, 45]. In this study, the Hartee-Fock method (method of approximation for the determination of the wave function and the energy of a quantum many-body system in a stationary state) was used to determinate both HOMO and LUMO orbitals (Figure 9 and Table 1) in Spartan'06 V112 program [46].

The results showed changes in both HOMO and LUMO values for the compound 9 compared with 10; this phenomenon could be conditioned by the difference in π orbitals density that is located in chemical structure 9 and 10.

Pharmacophore Ligand Model

For several years, some chemical models have been used to determine the three-dimensional orientation adopted by the functional groups of a molecule to predict its interaction with several biomolecules [46]; for example, the use of a pharmacophore model which can furnish a new insight to design novel molecules that can enhance or inhibit the function of a biological target which can be useful in new drug discovery. Analyzing this premise in this study, the LigandScout software [47] was used to develop a pharmacophore model for compounds 9 and 10 (Figure 10).

The results showed that functional groups involved in the compounds 9 and 10 could interact via hydrophobic contacts or as hydrogen bond acceptors or as hydrogen bond donor with some biomolecules.

Interaction Theoretical (Protein-Ligand)

Analyzing the hypothesis mentioned above and some reports which suggest that the formation of binary complexes between some compounds that act as ligands with several target biomolecules could induce changes in many activities of some biological systems [48-53]; Therefore, in this study, was carried out a theoretical analysis on the interaction of both compound 9 or 10 with Dwd3 protein using exemestane and anastrozole (aromatase inhibitors) [54] as a control in a Doc-

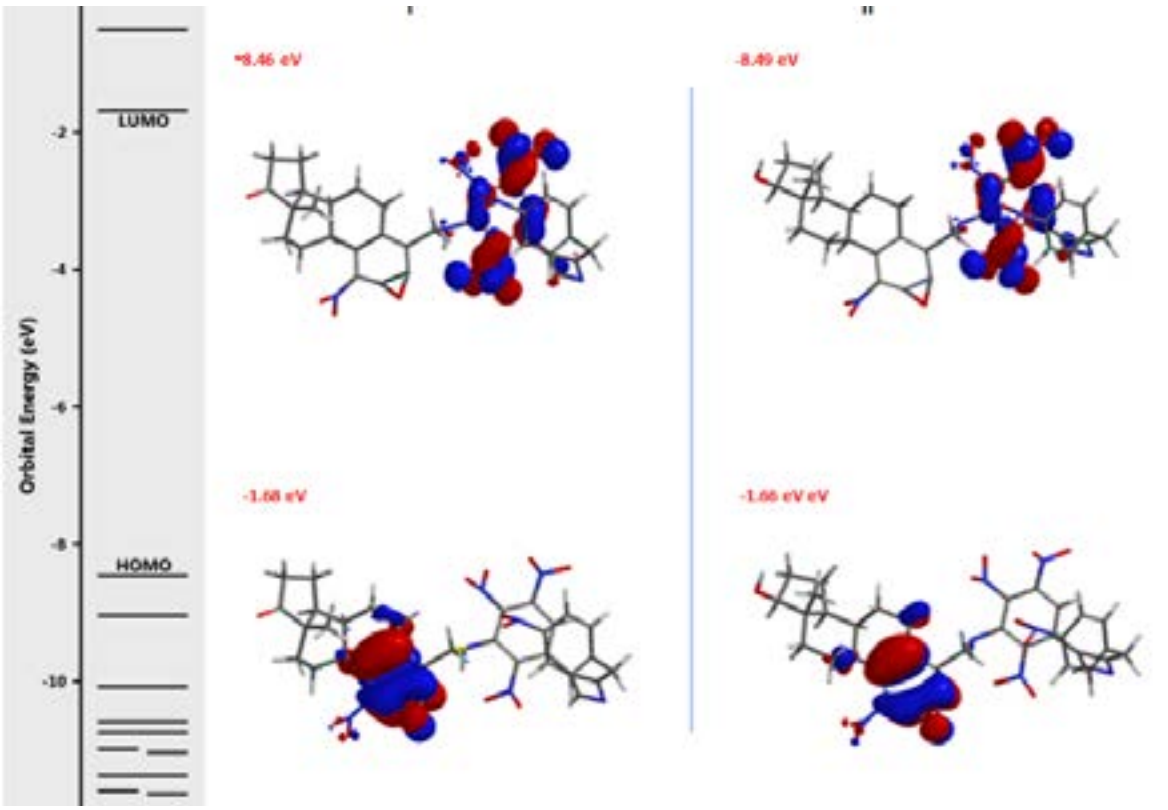

Figure 9. Molecular orbitals (HOMO and LUMO) involved in the compounds 9 (I) and 10 (II). Visualized with SPARTAN'06 software

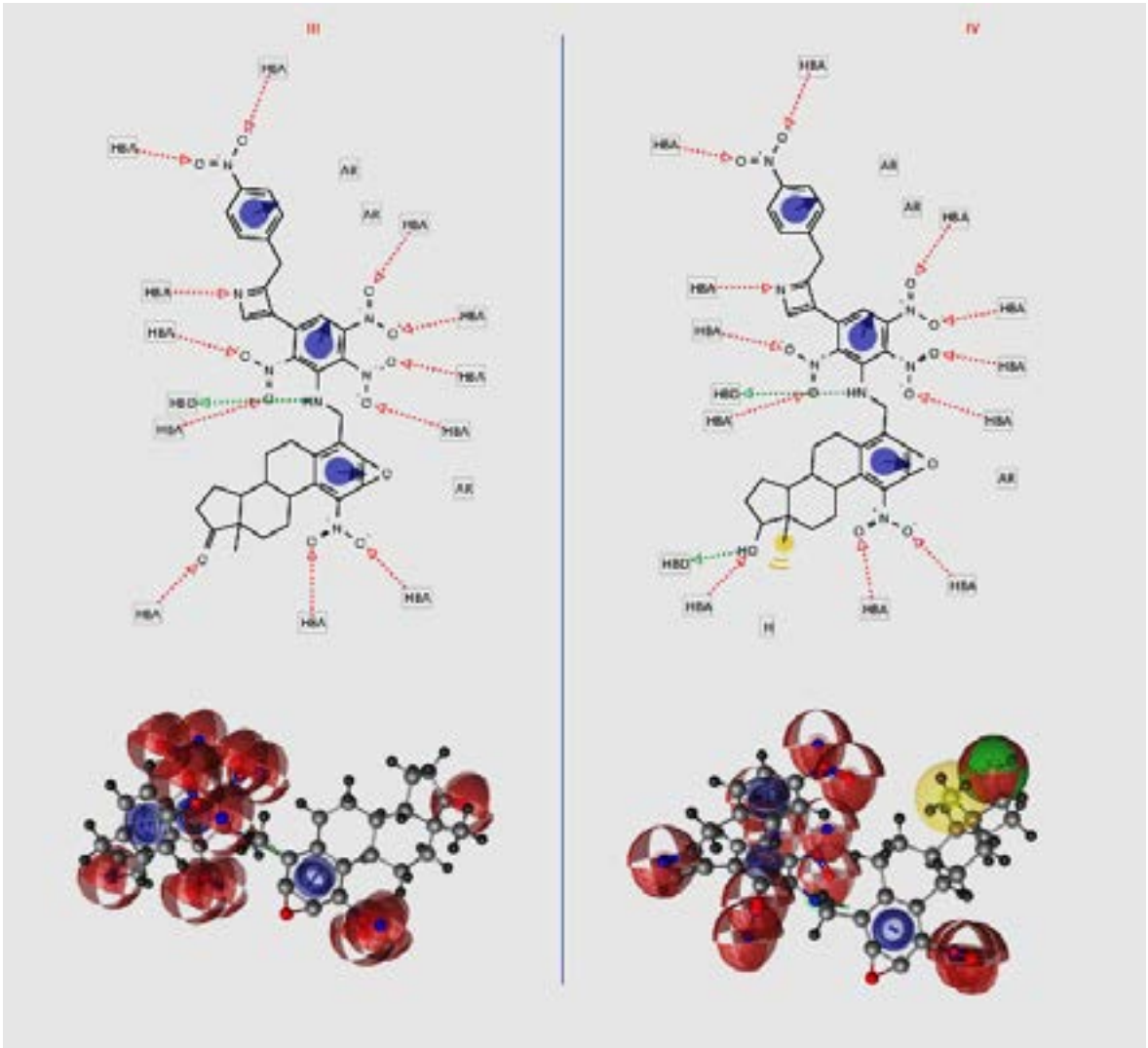

Figure 10. Theorethical pharmacophore from both compounds 9 (III) and 10 (1V) using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red) and hydrogen bond donor (HBD, green).

kingServer [23]; here, it is important to mention that Dockingserver was used because we believe that it is one of the most complete to analyze the protein-drug interaction.

The data (Table 2) showed differences in the interaction of both compounds 9 and 10 with some aminoacid residues involved in 2wd3 protein surface. In addition, other data suggest that there is another type of aminoacid residues in the interaction of both exemestane and anastrozole with 2Wd3. This phenomenon could be conditioned by the different conformations adopted by both compound 9 and 10 or the length of bound between the steroid-derivatives and the aminoacid residues involved in 2Wd3 protein surface. However, it is important to mention that some reports suggest that other thermodynamic factors such as free energy of binding, electrostatic energy, total intermolecular energy and Van der Waals (vdW) + hydrogen bond (Hbond) + desolvation energy can be involved in the interaction of several compounds with the proteins or enzymes [25, 26].

Thermodinamic Parameters

Analyzing the hypothesis above mentioned, in this study, some thermodynamic parameters were determinate using DockigServer [23]. Theoretical data (Table 3) indicates that there are differences in the thermodynamic parameters of exametane and anastrazole compared with both compounds 9 and 10. However, the theoretical results showed that inhibition constant (Ki)

involved in the interaction of both compounds 9 and 10 with 2WD3 protein surface was lower compared with exametane and anastrazole.

All these results suggest that both compounds 9 and 10 could exert a higher interaction with the 2Wd3 protein surface of, which can be translated as a decrease in aromatase activity.

CONCLUSIONS

In this study, is reported a facile synthesis of two steroid derivatives using several chemical strategies. In addition, the theoretical interaction of steroid derivatives (9 or 10) with the aromatase enzyme indicate that both compounds 9 and 10 could act as aromatase enzyme inhibitors which can be translated as good candidates for their evaluation in some cancer model; Nnevertheless, possibly the compound 10 could have higher activity against aromatase enzyme. However, to demonstrate

Table 2. Aminoacids residues involved in the interaction of anastrazole, exametane and compounds 9 (C-9) and 10 (C-10) with 2WD3 protein surface.

Anastrazole	Exametane	C-9	C-10
Gln_{92}	Trp_{5}	Trp_{5}	Trp_{5}
His_{94}	His_{64}	Leu_{60}	Asn_{62}
Val_{121}	Ala_{65}	Asn_{62}	Asn_{67}
Phe_{130}	His_{94}	His_{64}	Glu_{69}
Val_{134}	His_{119}	Glu_{69}	Ile_{91}
Leu_{140}	Val_{121}	His_{94}	Gln_{92}
Leu_{197}	Val_{142}	Phe_{130}	His_{94}
Thr_{199}	Leu_{197}	Thr_{199}	Val_{121}
Pro_{201}	Thr_{198}	Pro_{201}	Phe_{130}
	Thr_{199}		Pro_{201}
	Val_{206}		
	Trp_{208}		

Gln (glutamine); His (histidine); Val (valine); Leu (leucine); Thr (threonine); Pro (proline); Ala (alanine); Trp (tryphtophan); Asn (aspartic acid); Glu (glutamine); Phe (phenylalanine); Ile (Isoleucine).

Table 3. Thermodynamic parameters involved in the interaction of anastrazole, exametane and compounds 9 (C-9) and 10 (C-10) with 2WD3 protein surface.

Compound	Est. Free Energy of Binding (Kcal/mol)	Est. Inhi-bition Constant, Ki (μM)	cdW + Hbond + desolv Energy
Anastrazole	-5.11	180.06	-6.92
Exametane	-8.39	710.67	-8.36
C-9	-7.08	6.42	-7.11

Table 4. Thermodynamic factors involved in the interaction of anastrazole, exametane and compounds 9 (C_9) and 10 (C-10) with 2WD3 protein surface.

Compound	Electrost. Energy	Total Inter-molec. Energy	Interact. Surface
Anastrazole	-0.01	-6.92	666.43
Exametane	-0.03	-8.39	648.45
C-9	-0.01	-7.11	828.06
C-10	0.02	-8.83	927.79

this, experimental analyzes would have to be done in some biological model.

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CONFLICT OF INTEREST

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

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