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Synthesis and theoretical activity of three steroid-derivatives on both aromatase and 17β-hydroxysteroid dehydrogenase Type 1 enzymes

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ABSTRACT

Breast cancer is the most common malignancy in the worldwide. It is noteworthy, that several drugs are used for cancer breast; nevertheless, some these drugs can produce secondary effects such as changes in blood pressure, bone loss and others. The objective of this investigation was synthesizing three steroid derivatives (compounds **4**, **5** and **6**) to evaluate their theoretical activity against both aromatase (2W3D) and 17β-Hydroxysteroid dehydrogenase Type 1 (3BH4) enzymes using fisetin and exemestane as control in a docking model. The data found indicate that compound **5** could exert a greater interaction with the 2WD4 and 3BH4 proteins in comparison with fisetin, exemestane and compounds **4** or **6**. In conclusion, this compound could be a good candidate as both aromatase and 17β-hydroxysteroid dehydrogenase enzymes inhibitor.

Keywords: *Breast cancer, steroids, fisetin, exemestane and enzymes.*

1. INTRODUCTION

Several studies indicate that breast cancer is a risk factor to produce death in worldwide [1-3]; there are several drugs for the treatment of some of this disease such as tamoxifen (estrogen antagonist) [4], anastrozole, letrozole or exemetane (aromatase inhibitors) [5-7], fisetin or methyl paraben (17β-hydroxysteroid dehydrogenase type 1 inhibitors) [8, 9]; however some these drugs can produce secondary effects such as secondary endometrial cancer [10] and bone loss [11]. In the search a new therapeutic alternative, several compounds have been developed for treatment of breast cancer. For example, the 4-hydroxy-androstenedione derivative was prepared from androstenedione to evaluate their biological activity as an aromatase inhibitor using in human placenta [12]. In addition, a substituted pyrrolizine was prepared via reaction of 3-aryl-3-(pyrrol-1-yl)propionates with ethyl 4-(pyrrol-1-yl)-4-vinylbutyrate and their biological effect on aromatase enzyme was asses in human placenta [13]. A study, shown the preparation of 1,2,4-thiadiazoles from 4-methoxy-benzonitrile and 3-hydroxybenzonitrile as 17β-Hydroxysteroid dehydrogenase Type 1 inhibitors using a liver microsomes model [14]. Other study showed the synthesis of an androstan-17β-ol derivative from dihydrotestosterone as 17β-Hydroxysteroid dehydrogenase Type 10 inhibitor [15].

On the other hand, also, have been prepared some compounds to predict their biological activity against aromatase and 17β-Hydroxysteroid dehydrogenase using some theoretical models [16-17]. In this sense, some indenodiazine derivatives were synthesized from 2-methyl-5H-indeno[1,2-d]pyrimidine and N-bromosuccinimide to determinate its binding with aromatase using a 3D QSAR model [18]. Additionally, a dyhydroxyestratrienyl-acetate derivative was prepared from estradiol to predict their theoretical interaction with aromatase using 3D QSAR [19]. Recently was synthesized a new steroid derivative as 17β-Hydroxysteroid dehydrogenase Type 1 inhibitor using a theoretical model [20]. All these studies indicate that several drugs have prepared as inhibitors to both aromatase and 17β-hydroxysteroid dehydrogenase; however, some protocol used for their preparation use dangerous reagents and require specific conditions. Analyzing these data, the objective of this investigation was synthesize three steroid derivatives from estrone to evaluate its theoretical activity against both aromatase and 17β-hydroxysteroid dehydrogenase enzymes using some theoretical models.

2. MATERIALS AND METHODS

2.1. Chemical synthesis.

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.

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

In a round bottom flask (10 ml), formestane (200 mg, 0.66 mmol), phenylhydrazine (76 mg. 0.70 mmol) in 5 ml of acetic acid were stirred to reflux for 12 h. The solvent of the mixture produced was diminished to reduced pressure and purified through a crystallization using the methanol:hexane (4:1) system; yielding 67% of product; m.p. 72-74 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3402 and 1712: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.02 (s, 3H), 1.08-2.00 (m, 12H), 2.12-3.12 (m, 5H), 7.12-7.66 (m, 4H) 10.70 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 13.62, 18.90, 21.32, 21.70, 22.82, 30.84, 30.90, 33.72, 34.96, 35.62, 41.00, 47.52, 51.44, 53.70, 112.12, 116.90, 119.52, 119.78, 121.24, 121.60, 126.90, 132.32, 135.52, 149.56, 220.10 ppm. EI-MS m/z: 375.21. Anal. Calcd. for $C_{25}H_{29}NO_2$: C, 79.96; H, 7.78; N, 3.73; O, 8.52. Found: C, 79.90; H, 7.70.

(E)-N-((12aR,14aS)-12a,14a-dimethyl-1-oxo-1,2,3,3a,3b,4,5,7, 12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtha [2,1-b]carbazol-6-yl)acetimidic acid (3)

In a round bottom flask (10 ml), compound **2** (200 mg, 0.53 mmol) and 10 ml of acetonitrile were stirring to reflux for 12 h. The solvent of the mixture produced was diminished to reduced pressure and purified via a crystallization using the methanol:water (4:1) system; yielding 44% of product; m.p. 126-128 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3400, 3322 and 1710: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.04 (s, 3H), 1.06-1.88 (m, 10H), 1.96 (s, 3H), 2.00-7.26 (m, 7H), 7.24-7.40 (m, 2H), 7.60 (broad, 2H), 7.64, 7.72, (m, 2H). 13.62, 13.74, 19.22, 21.30, 21.67, 29.70, 30.82, 32.36, 34.90, 34.97, 35.54, 38.72, 47.52, 50.90, 51.44, 112.82, 118.78, 119.35, 121.15, 124.63, 126.00, 126.22, 129.12, 130.60, 137.34, 167.60, 220.10 ppm. EI-MS m/z: 416.24. Anal. Calcd. for $C_{27}H_{32}N_2O_2$: C, 77.85; H, 7.74; N, 6.73; O, 7.68. Found: C, 77.80; H, 7.70.

4-(cyanomethyl)phenyl(E)-N-((12aR,14aS)-12a,14a-dimethyl-1-oxo-1,2,3,3a,3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtho[2,1-b]carbazol-6-yl)acetimidate (4)

In a round bottom flask (10 ml), compound **3** (200 mg, 0.48 mmol) and (4-Nitro-phenyl)-acetonitrile (80 mg, 0.49 mmol), potassium carbonate (50 mg, 0.36 mmol) and 5 ml of dimethyl sulfoxide were stirring to room temperature for 72 h. The solvent of the mixture produced was diminished to reduced pressure and purified through a crystallization using the methanol:water (4:1) system; yielding 58% of product; m.p. 82-84 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3322, 2212, 1712, and 1212: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.04 (s, 3H), 1.06-2.12 (12H), 2.14 (s, 3H), 2.17-2.46 (m, 5H), 3.62 (m, 2H), 7.22 (m, 1H), 7.28 (m, 2H), 7.38 (m, 1H), 7.54 (m, 2H), 7.66-7.94 (m, 3H) ppm. 13.62, 19.20, 20.80, 21.32, 21.70, 23.42, 29.70, 30.86, 32.38, 34.89, 34.97, 35.58, 38.72, 47.51, 50.90, 51.44, 112.83, 117.40, 119.32, 120.23, 120.32, 121.12, 124.52, 124.60, 127.00, 127.32, 128.57, 128.54, 130.60, 132.12, 138.30, 156.30, 178.71, 220.10 EI-MS m/z: 531.28. Anal. Calcd. for $C_{35}H_{37}N_3O_2$: C, 79.06; H, 7.01; N, 7.90; O, 6.02. Found: C, 79.00; H, 7.00.

4-hydroxybenzoic(E)-N-((12aR,14aS)-12a,14a-dimethyl-1-oxo-1,2,3,3a,3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtho[2,1-b]carbazol-6-yl)acetimidic anhydride (5)

In a round bottom flask (10 ml), compound **3** (200 mg, 0.48 mmol) and 4-hydroxybenzoic acid (69 mg, 0.50 mmol), potassium carbonate (50 mg, 0.36 mmol) and 5 ml of dimethyl sulfoxide were stirred to room temperature for 72 h. The solvent of the mixture produced was diminished to reduced pressure and purified via a crystallization using the methanol:hexane:water (4:1:1) system; yielding 58% of product; m.p. 126-128 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3400, 3320, 1742, and 1712: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.04 (s, 3H), 1.06-2.12 (12H), 2.14 (s, 3H), 2.17-2.46 (m, 5H), 7.02 (m, 2H), 7.28-7.74 (m, 4H), 8.30 (m, 2H), 9.00 (broad, 2) ppm. 13.62, 19.20, 20.62, 21.32, 21.70, 29.72, 30.88, 32.38, 34.92, 34.94, 35.60, 38.72, 47.52, 50.90, 51.44, 112.82, 117.62, 119.32, 119.72, 121.12, 123.95, 124.62, 125.50, 126.82, 128.74, 131.04, 134.08, 138.12, 160.22, 161.90, 171.44, 220.10 ppm. EI-MS m/z: 536.26. Anal. Calcd. for $C_{34}H_{36}N_2O_4$: C, 76.09; H, 6.76; N, 5.22; O, 11.93. Found: C, 76.00; H, 6.70.

benzoic(E)-N-((12aR,14aS)-12a,14a-dimethyl-1-oxo-1,2,3,3a, 3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6] naphtho[2,1-b]carbazol-6-yl)acetimidic anhydride (6)

In a round bottom flask (10 ml), compound **3** (200 mg, 0.48 mmol) and benzoic acid (60 mg, 0.49 mmol), potassium carbonate (50 mg, 0.36 mmol) and 5 ml of dimethyl sulfoxide were stirred to room temperature for 72 h. The solvent of the mixture produced was diminished to reduced pressure and purified via a crystallization using the methanol:water (4:1) system; yielding 58% of product; m.p. 114-116 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3322, 1742, and 1710: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 1.04 (s, 3H), 1.06-2.12 (12H), 2.14 (s, 3H), 2.17-2.46 (m, 5H), 7.28-7.40 (m, 2H), 7.56-7.60 (m, 3H), 7.66-7.74 (m, 2H), 8.36 (m, 2H), 9.92 (broad, 1) ppm. 13.62, 19.20, 20.62, 21.32, 21.70, 29.72, 30.88, 32.38, 34.92, 34.94, 35.60, 38.72, 47.52, 50.90, 51.44, 112.82, 117.62, 119.32, 119.72, 121.12, 124.62, 125.50, 126.82, 128.74, 130.60, 131.04, 131.12, 132.96, 133.50, 138.12, 160.22, 171.44, 220.10 ppm. EI-MS m/z: 520.27. Anal. Calcd. for $C_{34}H_{36}N_2O_3$: C, 78.43; H, 6.97; N, 5.38; O, 9.22. Found: C, 78.38; H, 6.90.

2.2. Physicochemical parameters evaluation. Some electronic factors of compounds **4**, **5** and **6** such as M_V (molar volume), M_R (molar refractivity), 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 both ACD/Chem Sketch and Spartan'06 programs [21, 22].

2.3. Pharmacophore evaluation. The 3D pharmacophore method for the compounds **4**, **5** and **6** was determinate using LigandScout 4.08 software [23, 24].

2.4. Theoretical evaluation. The interaction of compounds **4** to **6** with both aromatase (2WD3) and 17β-Hydroxysteroid dehydrogenase Type 1 (3HB4) enzymes [25, 26] was developed using a Docking model [27].

3. RESULTS

Chemical synthesis.

In this study, three steroid derivatives were prepared using some chemical strategies:

First stage. This step involved the preparation of an indol-steroid derivative (compound **2**); it is noteworthy that several indol analogs have been synthesized using some reagents such as *N*-chlorosuccinimide [28], gold [29], rhodium [30], CuBr [31] and others. In this study, the compound **2** was prepared from formestane and diphenylhydrazine (Figure 1).

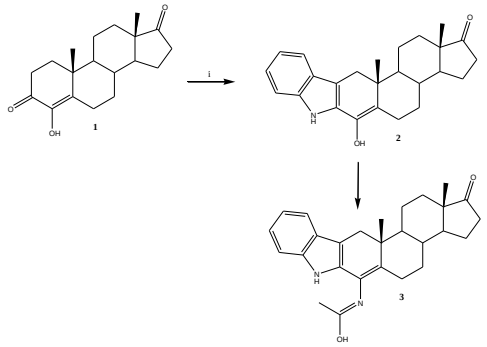

Figure 1. Preparation of an oxo-tetradecahydrocyclopenta-carbazol-acetimidic acid derivative (**3**). Reaction of formestane (**1**) with phenylhydrazine (i) to form a dodecahydrocyclopenta-carbazolone (**2**). Then, compound 2 reacted with acetonitrile for the synthesis of **3**.

The ^{1}H NMR spectra for **2** showed several signals at 0.90-1.08 ppm for both methyl groups; at 1.08-3.12 ppm for steroid moiety; at 7.12-7.60 ppm for indol group; at 10.70 ppm for both hydroxyl and amino groups (Figure 2). ^{13}C NMR spectra for **2** showed several signals at 13.62-18.90 ppm for both methyl groups bound to steroid nucleus; at 21.32-53.50, 121.52 and 149.56 ppm for steroid moiety; at 112.12-121.24 and 126.90-135.52 ppm for indol ring; at 220.10 ppm for ketone group. Finally, the mass spectrum from **2** showed a molecular ion (m/z) 375.21.

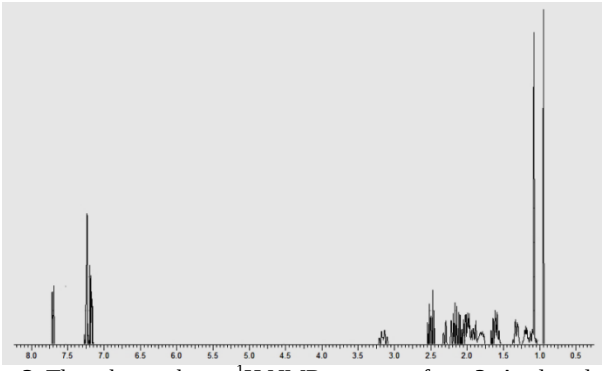

Figure 2. The scheme shown ^{1}H NMR spectrum from **2**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in CDCl3. Axis abscissa (ppm); ppm = parts per million.

Second stage. Some reports have showed the preparation of several acetimidic acid derivatives using some reagents such as 2-bromocyclohexylacetimidium chloride [32], *N*-benzyl-*N*-nitro-sopivalamide [33], *o*-aminobenzoylhydrazine [34]. Analyzing these data and a report which showed the synthesis of a steroid-ethanimidic acid derivative [35]; in this study an azahexacyclotetracosa-ethanimidic acid (**3**) was prepared from compound **2** and acetonitrile (Figure 1). The ^{1}H NMR spectra for **3** (Figure 3) showed several signals at 0.90-1.04 for both methyl groups bound to steroid nucleus; at 1.96 ppm for methyl group bound to imino group; at 1.06-1.88 and 2.00-7.26 ppm for steroid moiety; at 7.24-7.40 and 7.64-7.72 ppm for indol group; at 7.60 ppm for both hydroxyl and amino groups (Figure 3). ^{13}C NMR spectra for **3** showed several signals at 13.62 and 19.22 ppm for methyl groups bound to steroid nucleus; at 13.74 ppm for methyl bound to imino group; at 21.30-51.44 and 126.22-129.12 ppm for steroid moiety; at 112.82-126.00 and 130.60-137.34 ppm for indol group; at 167.60 ppm for imino group; at 220.10 ppm for ketone group. Additionally, the mass spectrum from **3** showed a molecular ion (m/z) 416.25.

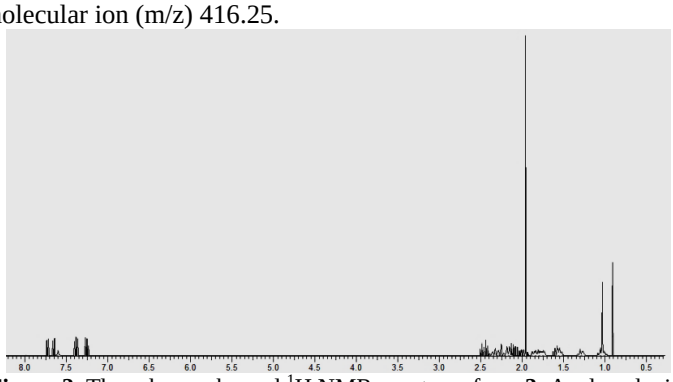

Figure 3. The scheme showed ^{1}H NMR spectrum from **3**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in CDCl3. Axis abscissa (ppm); ppm = parts per million.

Third stage. In this stage, some ester derivatives (Figure 4) were prepared using a previously method reported for ester-steroid derivatives via esterification of hydroxyl group [36]. In this study, the compound **3** reacted with (4-Nitro-phenyl)-acetonitrile or 4-hydroxybenzoic acid or benzoic acid to form the ester derivatives (compounds **4** or **5** or **6**).

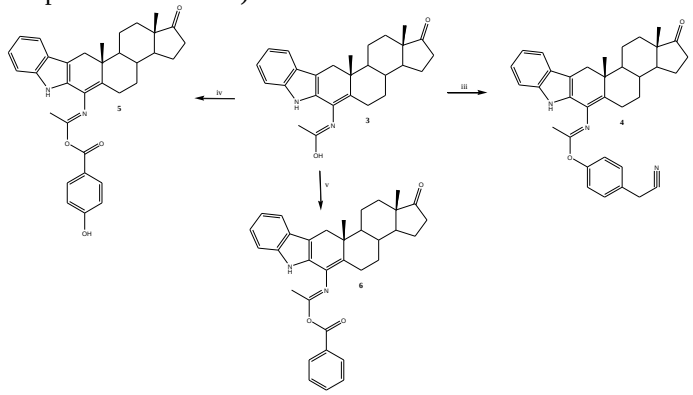

Figure 4. Synthesis of three steroid derivatives (**4** or **5** or **6**). Reaction of **3** with (4-Nitro-phenyl)-acetonitrile (iii) to form a 4-(cyanomethyl)phenyl-tetradecahydrocyclopenta-carbazol-acetimidate (**4**). Then, **3** reacted with 4-hydroxy benzoic acid (iv) for the synthesis of oxo-tetradecahydrocyclopen- ta-carbazol-acetimidic anhydride (**5**).

Finally, **6** was prepared via reaction of **3** with benzoic acid (v). The ^{1}H NMR spectra for **4** showed several signals at 0.90-1.04 for both methyl groups bound to steroid nucleus; at 2.14 ppm for methyl group bound to imino group; at 1.06-2.12 and 2.17-2.46 ppm for steroid moiety; at 3.62 ppm for methylene group bound to both phenyl and cyanide groups; at 7.22, 7.38 and 7.66-7.94 ppm for indol group; at 7.28 and 7.54 ppm for phenyl group bound to ester group (Figure 5). ^{13}C NMR spectra for **4** showed several signals at 13.62-19.24 ppm for both methyl groups bound to steroid nucleus; at 20.80 ppm for methyl bound to imino group; at 21.32-21.70, 29.70-52.44, 12.32 and 130.60 ppm for steroid moiety; at 23.42 ppm for methylene bound to both phenyl and cyanide groups; at 112.83, 119.32-120.23, 121.12, 124.60-127.00 and 132.12-138.30 ppm for steroid moiety; at 117.40 ppm for cyanide group; at 120.32, 124.52, 128.54 and 156.30 ppm for phenyl group bound to ester group; at 178.70 ppm for imino group; at 220.10 ppm for ketone group. Finally, the mass spectrum from **4** showed a molecular ion (m/z) 531.28.

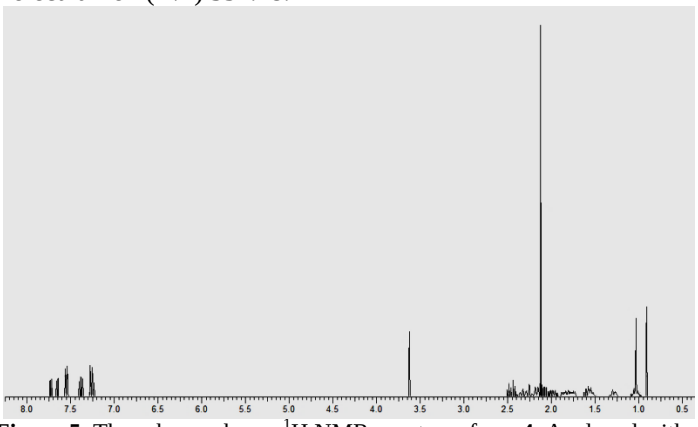

Figure 5. The scheme shown ^{1}H NMR spectrum from **4**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in CDCl3. Axis abscissa (ppm); ppm = parts per million.

Other data showed several signals involved in ^{1}H NMR spectra for compound **5** at 0.90-1.04 for both methyl groups bound to steroid nucleus; at 2.14 ppm for methyl group bound to imino group; at 1.06-2.12 and 2.17-2.46 ppm for steroid moiety; at 7.02-8.30 ppm for phenyl group bound to ester group; at 7.28-7.74 ppm for indol group; at 9.00 ppm for both hydroxyl and amino groups (Figure 6).

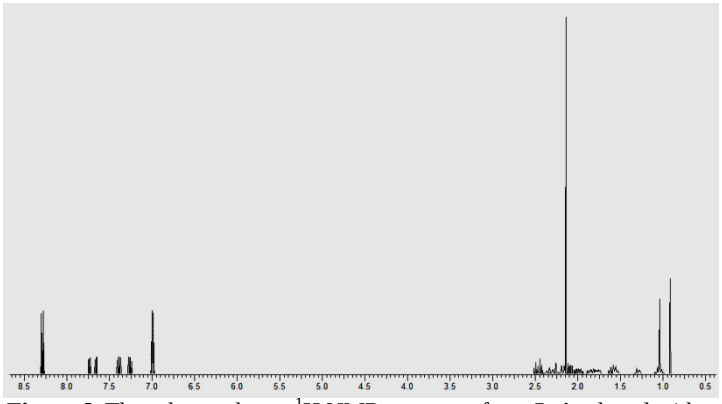

Figure 6. The scheme shown ^{1}H NMR spectrum from **5**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in CDCl3. Axis abscissa (ppm); ppm = parts per million.

^{13}C NMR spectra for **5** showed several signals at 13.62-19.20 ppm for both methyl groups bound to steroid nucleus; at 20.62 ppm for methyl bound to imino group; at 21.32-51.44, 125.50 and 131.04 ppm for steroid moiety; at 112.82, 119.32-121.12, 124.62, 126.82 and 138.12 for indol group; at 117.62, 123.95, 134.08 and 161.90 ppm for phenyl group bound to ester group; at 160.22 ppm for imino group; at 171.44 ppm for ester group; at 220.10 ppm for ketone group. Additionally, the mass spectrum from **5** showed a molecular ion (m/z) 536.26.

Finally, the ^{1}H NMR spectra for **6** showed several signals at 0.90-1.04 ppm for both methyl groups bound to steroid nucleus; at 2.14 ppm for methyl group bound to imino group; at 1.06-2.12 and 2.17-2.46 ppm for steroid moiety; at 7.28-7.40 and 7.76-7.74 ppm for indol group; at 7.56-7.60 and 8.36 ppm for phenyl group bound to ester group; at 9.92 ppm for amino group (Figure 7). ^{13}C NMR spectra for **6** showed several signals at 13.62-19.20 ppm for both methyl groups bound to steroid nucleus; at 20.62 for methyl bound to imino group; at 21.32-51.44, 125.50 and 131.04 ppm for steroid moiety; at 112.82-124.62, 126.82-128.74 and 138.12 ppm for indol group; at 130.60 and 131.12-135.50 ppm for phenyl group bound to ester group; at 160.22 ppm for imino group; at 171.44 ppm for ester group; at 220.10 ppm for ketone group. Finally, the mass spectrum from **6** showed a molecular ion (m/z) 520.27.

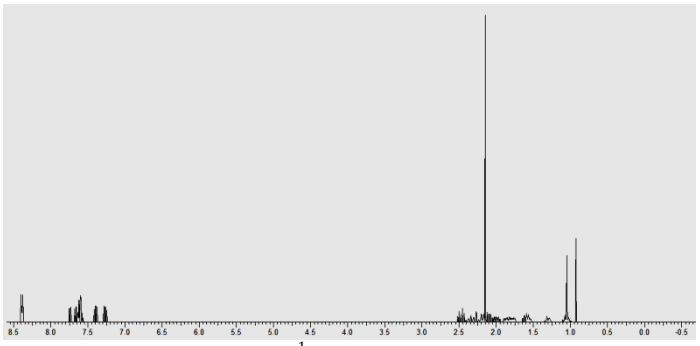

Figure 7. The scheme showed ^{1}H NMR spectrum from **6**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in CDCl3. Axis abscissa (ppm); ppm = parts per million.

Electronic parameters. The levels of energy (HOMO and LUMO) for compounds 3 to 6 were evaluated using SPARTAN´06 software (Figure 8 and 9), with Hartee-Feck method at 321-G level [22]. The theoretical data showed that LUMO was higher for the compound **3** compared with the compounds **2**, 4, **5** and **6** (Figure 8 and 9); this phenomenon may condition another type of physicochemical factors related with their chemical structure in this study such as molar refractory (M_V) and molar refractory (M_R) [37].

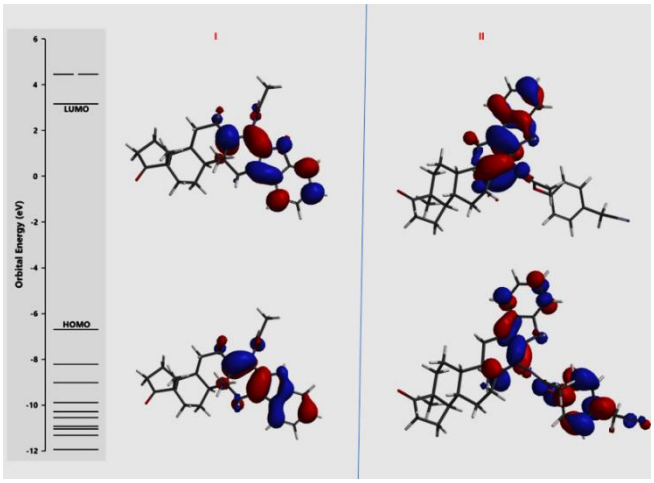

Figure 8. In the scheme are shown both HOMO and LUMO for either **3** (I) or **4 (**II**)**. The figure was visualized with SPARTAN´06 software.

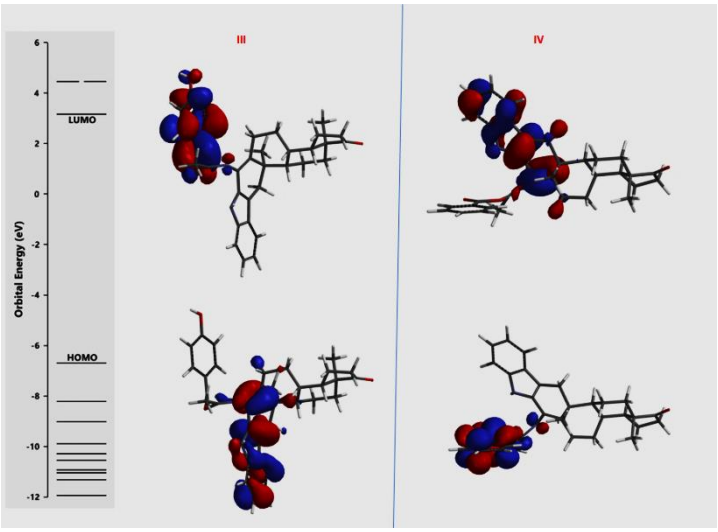

Figure 9. In the scheme are shown both HOMO and LUMO for either **5** (III) or **6 (**IV**)**. The figure was visualized with SPARTAN´06 software.

Physicochemical parameters of both compounds 3 and 4. Analyzing these data in this investigation, both M_V and M_R descriptors were determinate using ChemSketch 3.5 program [21]. Theoretical data showed that M_V and M_R were higher for **4** compared with **2**, **3**, **5** and **6** (Table 1).

Table 1. Physicochemical parameters involve in the structure of compounds **2-6**.

Parameter	2	3	4	5	6
Molar Refractivity (cm^3)	109.83	120.00	156.78	151.96	151.11
Molar Volume (cm^3)	295.20	307.90	413.60	395.30	398.10
Polarizability (cm^3)	43.54	74.60	84.71	83.82	83.43
Parachor (cm^3)	820.01	826.|0	1100.80	1066.40	1060.70
Index of refraction	1.66	1.70	1.68	1.69	1.68
Surface Tension (dyne/cm)	59.50	51.80	50.10	52.90	50.40
PSA $Å^2$	45.72	52.71	55.61	74.41	51.05
Density g/cm^3	1.27	1.35	1.28	1.35	1.30
HBD	2	2	1	2	1
HBA	2	4	5	5	4
HOMO (eV)	-6.54	-6.70	-6.86	-6.92	-6.91
LUMO (eV)	2.77	3.17	2.91	2.51	2.10

These results suggest that higher volume translated as steric hindrance and different conformational changes might be two factors which influence on biological activity exerted by **4** compared with the other compounds involved in this study.

Nevertheless, it is noteworthy that also other physiochemical factors such as hydrogen bond donor groups (HBD) and hydrogen bond acceptor groups (HBA), topological polar surface area (TPSA) can produce changes on biological activity of some compounds in several theoretical models [38]. Therefore, these factors involved in the chemical structure of compounds 2-4 were determinate using LigandScout software [23, 24]. The theoretical results showed values of <10 for compounds **2** to **6**; These data indicate that these compounds could have good absorption and permeability on plasma membranes, which could be translated as changes on the biological activity of some system as described by Lipinski's rule [39].

Pharmacophore evaluation. Some studies have showed that the pharmacophore model can be used to design new molecules with pharmacological activity [23, 24]. In this way, the LigandScout software was used to develop two pharmacophores from compounds **4** to **6**. The theoretical data (Figures 10 and 11) showed the different types of functional groups that can act as hydrogen bond receptors or as hydrogen bond donors with some biomolecules.

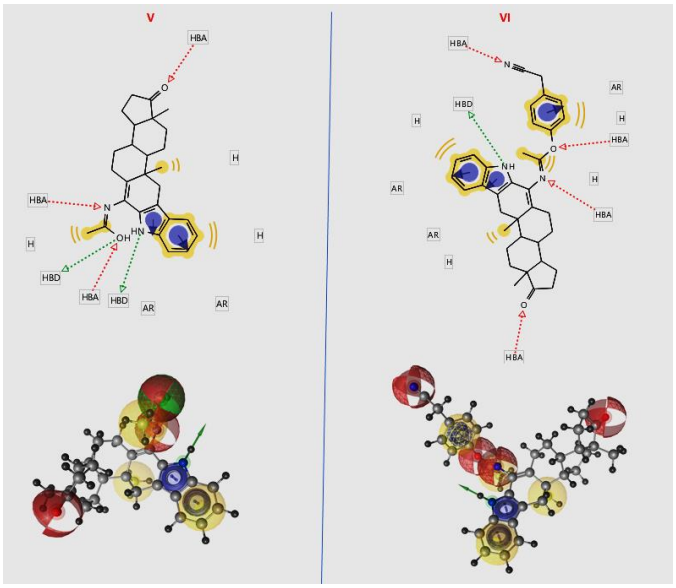

Figure 10. Scheme represents a pharmacophore from both compounds **3** (V) and **4** (VI) using the LigandScout software. The model involves a hydrogen bond acceptor (HBA, red) and hydrogen bond donor (HBD, green).

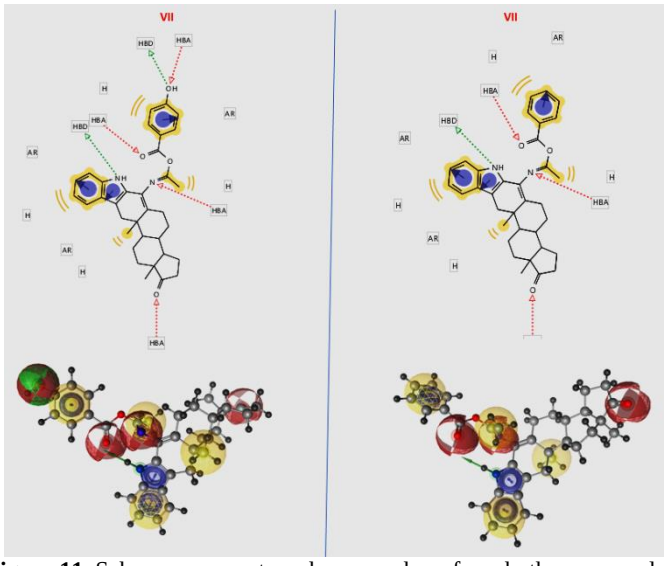

Figure 11. Scheme represents a pharmacophore from both compounds **5** (VII) and **6** (VIII) using the LigandScout software. The model involves a hydrogen bond acceptor (HBA, red) and hydrogen bond donor (HBD, green).

Interaction theoretical (protein-ligand). Since several years ago, several theoretical methods were used to evaluate the binding of some drugs with protein or enzymes [40]. Therefore, in this study was carried out a theoretical analysis on interaction of compound **4-6** with either 2WD3 and 3HB4 proteins in a Docking model [27] using both exemestane and fisetin as controls. The data (Table 2) showed differences in the interaction of exemestane (aromatase inhibitor) and compound **2-6** on 2W3D protein surface.

Table 2. Aminoacids residues involved in the interaction of exemestane and compounds **4-6** with 2WD3 protein surface.

	Exametane	C-4	C-5	C-6
Aminoacid Residues	Trp_{5} His_{94} His_{96} His_{119} Val_{121} Val_{142} Leu_{197} Thr_{198} Thr_{199} Val_{206} Trp_{208}	Trp_{5} Asn_{62} His_{64} Asn_{67} Glu_{69} Ile_{91} Gln_{92} His_{94} Val_{121} Phe_{130} Leu_{197} Thr_{198} Thr_{199}	Trp_{5} Asn_{62} His_{64} Asn_{67} Glu_{69} Ile_{91} Gln_{92} Val_{121} Phe_{130} Leu_{197} Thr_{199} Pro_{201}	Trp_{5} Asn_{62} His_{64} Asn_{67} Glu_{69} Ile_{91} Gln_{92} His_{94} Val_{121} Phe_{130} Leu_{140} Val_{142} Leu_{197} Thr_{198} Thr_{199} Pro_{201} Val_{206}

To evaluate if these differences might depend on their functional groups, the distance involved between the compounds **4** to **6** and 2WD3 protein surface was determinate using the SeeSAR program (Table 3).

Theoretical results showed that both ester and imino group could be responsible for a higher interaction with 2WD3 protein surface and possibly with another type of enzymes. To evaluate this hypothesis, the interaction of compounds **4** to **6** with the 3HB4 protein surface was determined using fisetin as a control. The results (Table 4) showed that several differences in the interaction of fisetin and compounds **4-6** with aminoacid residues involved in the 3HB4 protein surface.

Table 3. Distance involved in the interaction between compounds **4** to **6** with 2WD3 protein surface.

Comp.	Ketone (Å)	Cyanide [Å]	Imino [Å]	Indole [Å]	Hydroxyl [Å]	Ester [Å]
4	Trp_{140} (17.35)	Trp_{140} (6.99) Ser_{142} (6.38)	Ser_{12} (13.67) Leu_{149} (16.96) Asn_{152} (16.40) Tyr_{155} (18.50) Lys_{159} (2.79) Cys_{185} (11.11) Pro_{187} (2.79) Phe_{192} (10.34)	Leu_{149} (16.87) Tyr_{218} (22.14) Ser_{222} (25.58)	Leu_{149} (13.80) Ser_{142} (12.31) Asn_{152} (14.73)	-
5	Ser_{12} (13.98)	-	Tyr_{155} (11.90) Cys_{185} (17.79)	Cys_{185} (20.69)	Leu_{96} (14.10) Asn_{152} (12.84) Lys_{159} (2.72) Phe_{192} (9.28)	Ser_{12} (19.89) Leu_{96} (14.74) Asn_{152} (13.37) Tyr_{155} (9.18) Lys_{159} (6.53) Met_{193} (13.37)
6	Ser_{142} (15.44) Tyr_{155} (15.44) Cys_{185} (20.51)	-	Ser_{12} (22.27) Leu_{96} (16.88) Ser_{142} (11.53) Tyr_{155} (11.53) Lys_{159} (7.65) Phe_{192} (16.09)	Ser_{142} (25.90)		Ser_{12} (19.89) Leu_{96} (13.31) Ser_{142} (10.66) Asn_{152} (12.22) Lys_{159} (5.58) Cys_{185} (15.32)

Table 4. Aminoacids residues involved in the interaction of exametane and compounds **4-6** with 3BH4 protein surface.

	Fisetin	C-4	C-5	C-6
Aminoacid Residues	Trp_{5} His_{94} His_{96} His_{119} Val_{121} Val_{142} Leu_{197} Thr_{198} Thr_{199} Val_{206} Trp_{208}	Trp_{5} Asn_{62} His_{64} Asn_{67} Glu_{69} Ile_{91} Gln_{92} His_{94} Val_{121} Phe_{130} Leu_{197} Thr_{198} Thr_{199}	Trp_{5} Asn_{62} His_{64} Asn_{67} Glu_{69} Ile_{91} Gln_{92} Val_{121} Phe_{130} Leu_{197} Thr_{199} Pro_{201}	Trp_{5} Asn_{62} His_{64} Asn_{67} Glu_{69} Ile_{91} Gln_{92} His_{94} Val_{121} Phe_{130} Leu_{140} Val_{142} Leu_{197} Thr_{198} Thr_{199} Pro_{201} Val_{206}

In addition, the distance between the compounds 4 to 6 with the aminoacid residues involved on 3HB4 protein surface was determinate. The results showed that the imino group could have greater specificity in the interaction between compounds 4-6 with the 3BH4 protein surface (Table 5).

Table 5. Distance involved in the interaction between compounds **4** to **6** with 3BH4 protein surface.

Comp.	Ketone (Å)	Cynide [Å]	Imino [Å]	Indol [Å]	Hydroxyl [Å]	Ester [Å]
4	Trp_{140} (17.35)	Trp_{140} (6.99) Ser_{142} (6.38)	Ser_{142} (13.67) Leu_{149} (16.96) Asn_{152} (16.40) Tyr_{155} (18.50) Lys_{159} (2.79) Cys_{185} (11.11) Pro_{187} (2.79) Phe_{192} (10.34)	Leu_{149} (16.87) Tyr_{218} (22.14) Ser_{222} (25.58)	Leu_{149} (13.80) Ser_{142} (12.31) Asn_{152} (14.73)	-
5	Ser_{12} (13.98)	-	Tyr_{155} (11.90) Cys_{185} (17.79)	Cys_{185} (20.69)	Leu_{96} (14.10) Asn_{152} (12.84) Lys_{159} (2.72) Phe_{192} (9.28)	Ser_{12} (19.89) Leu_{96} (14.74) Asn_{152} (13.37) Tyr_{155} (9.18) Lys_{159} (6.53) Met_{193} (13.37)
6	Ser_{142} (15.44) Tyr_{155} (15.44) Cys_{185} (20.51)	-	Ser_{12} (22.27) Leu_{96} (16.88) Ser_{142} (11.53) Tyr_{155} (11.53) Lys_{159} (7.65) Phe_{192} (16.09)	Ser_{142} (25.90)		Ser_{12} (19.89) Leu_{96} (13.31) Ser_{142} (10.66) Asn_{152} (12.22) Lys_{159} (5.58) Cys_{185} (15.32)

Nevertheless, it is noteworthy that some reports suggest that other thermodynamic factors; for example, 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 some proteins or enzymes [25, 26]. Therefore, in this study, these

thermodynamic parameters were determinate using DockigServer [27]. Theoretical data (Table 6 and 7) indicate that there are differences in the thermodynamic factors values of exametane and fisetin compared with compounds **4** to **6**.

Table 6. Thermodynamic parameters involved in the interaction of exametane and compounds **4-6** with 2WD3 protein surface.

Compound	Est. Fee Energy of Binding (kcal/mol)	Est. Inhibition Constant, Ki (µM)	cdW + Hbond + desolv Energy	Electrost. Energy	Total Intermolec. Energy	Interact. Surface
Exametane	-7.33	4.25	-7.36	0.03	-7.33	688.06
4	-8.77	374.92	-10.47	0.06	-10.42	981.15
5	-7.64	2.51	-9.19	0.03	-9.16	909.54
6	-7.02	7.17	-8.34	0.01	-8.32	880.11

Other theoretical results showed that inhibition constant (Ki) of the compounds **5** with 2WD3 protein surface was lower compared with exemestane and compounds 4 or **6** (Table 6). In addition, the theoretical data showed in Table 7 indicated that Ki value involved in the binding between the compound 5 with 3BH4 protein surface was lower compared to fisetin and compounds **4** or **6**. All these results suggest that compounds **4** to **6** could interact with both 2DW3 and 3BH4 proteins. However, compound 5 could exert a higher interaction with these enzymes, which translates into the possibility of high enzymatic inhibition.

Table 7. Thermodynamic factors involved in the interaction of compounds **4-6** and fisetin with with 3BH4 protein surface.

Compound	Est. Fee Energy of Binding (kcal/mol)	Est. Inhibition Constant, Ki (µM)	cdW + Hbond + desolv Energy	Electrost. Energy	Total Inter-molec. Energy	Interact. Surface
Fisetin	-7.20	5.24	-7.69	-0.06	-7.75	709.06
4	-11.04	8.10	-12.32	0.16	-12.16	1140.94
5	-11.60	3.15	-12.76	-0.07	-12.83	1094.61
6	-2.90	7.47	-3.23	-0.07	-3.29	1102.41

4. CONCLUSIONS

In this investigation a facile synthesis of three steroid-derivatives was reported. In addition, theoretical study suggests that compound **5** could be good candidate as inhibitor of the biological activity of both aromatase and 17β-hydroxysteroid dehydrogenase enzymes which is translated such as a possible drug for treatment of breast cancer.

5. REFERENCES

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