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 can be 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-methoxybenzonitrile 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 dihydroxyestratrienylacetate 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. 1H and 13C NMR (nuclear magnetic resonance) spectra were determine with a Varian VX300/5 FT NMR spectrometer at 300 MHz (megahertz) in CDCl3 (deuterated...
In a round bottom flask (10 ml), forsmene (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 °C; IR (V_N, cm^-1) 3430, 3402 and 1712; ^1H NMR (500 MHz, Chloroform-d) δH: 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. ^13C NMR (75.4 Hz, CDCl3) δC: 13.62, 18.90, 21.32, 21.70, 22.82, 30.84, 30.90, 33.72, 34.96, 36.52, 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. Calcld. for C23H28N2O: C, 79.96; H, 7.78; N, 3.73; O, 8.52. Found: C, 79.90; H, 7.70.

In a round bottom flask (10 ml), compound 2 (200 mg, 0.53 mmol) and 10 ml of acetonitrile were stirring for reflux to 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. 127.00, 127.32, 128.54, 130.60, 132.12, 138.30, 156.30, 178.71, 220.10 EI-MS m/z: 531.28. Anal. Calcld. for C38H37NO4: C, 79.06; H, 7.01; N, 7.90; O, 6.02. Found: C, 79.00; H, 7.00.

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 °C; IR (V_N, cm^-1) 3430, 3400, 3320, 1742, and 1712; ^1H NMR (500 MHz, Chloroform-d) δH: 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. Calcld. for C39H36N2O4: C, 76.09; H, 6.76; N, 5.22; O, 11.93. Found: C, 76.00; H, 6.70.

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 °C; IR (V_N, cm^-1) 3430, 3322, 1742, and 1710; ^1H NMR (500 MHz, Chloroform-d) δH: 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, 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: 520.27. Anal. Calcld. for C38H37NO4: 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_e (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].
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).

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.

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-aminobenzoylehydrazine [34]. Analyzing these data and a report which showed the synthesis of a steroid-ethanimidic acid derivative [35]; in this study an azahexacyclotetraconta-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 group 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.

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 1. Preparation of an oxo-tetradecahydrcyclopenta-carbazol-acetimidic acid derivative (3). Reaction of formestane (1) with phenylhydrazine (i) to form a dodecahydrocyclopenta-carbazolone (2).

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.

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.

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-tetradecahydrcyclopenta-carbazol-acetimidate (4). Then, 3 reacted with 4-hydroxy benzoic acid (iv) for the synthesis of oxo-tetradecahydrocyclopenta-carbazol-acetimidic anhydride (5).
Finally, 6 was prepared via reaction of 3 with benzoic acid (v). The 1H 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). 13C 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 22.01 ppm for indol group; at 7.28 and 7.54 ppm for phenyl group bound to ester group; at 138.12 ppm for indol group; at 7.28 and 7.54 ppm for phenyl group bound to ester group; at 130.60 and 131.12 ppm for phenyl group bound to ester group; at 178.70 ppm for imino group; at 220.10 ppm for ketone group. Additionally, the mass spectrum from 5 showed a molecular ion (m/z) 536.26.

Finally, the 1H 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). 13C 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.

Other data showed several signals involved in 1H NMR spectra for compound 5 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 7.02-7.28 ppm for methyl group bound to imino group; at 1.06 and 1.20 ppm for phenyl group bound to ester group; at 2.12 and 2.17 ppm for steroid moiety; at 7.28-7.74 ppm for indol group; at 9.00 ppm for both hydroxyl and amino groups (Figure 6).

13C 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 ppm 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 1H 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). 13C 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.
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| 826.80| 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 physicochemical 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.
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 determined 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     | Ser19 (13.98) | -           | Ty113 (11.90) | Cys185 (17.79) | Cys185 (20.69) | Let94 (14.10) | Ser19 (19.89) | Leu96 (14.74) | Ser19 (13.37) | - |
| 5     | Ser19 (15.44) | Ty113 (15.14) | Cys185 (20.51) | Ser19 (25.90) | Ser19 (19.89) | Leu96 (13.31) | Ser19 (10.66) | Asn52 (2.72) | Ser19 (5.58) | Cys185 (15.32) |
| 6     | Ser19 (22.27) | Leu96 (16.88) | Ser19 (11.53) | Ty113 (11.53) | Cys185 (7.65) | Phe107 (16.19) | Ser19 (25.90) | Ser19 (19.89) | Leu96 (13.31) | Ser19 (10.66) | Asn52 (2.72) | Ser19 (5.58) | Cys185 (15.32) |

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

Table 4. Aminoacid residues involved in the interaction of exametane and compounds 4-6 with 3HB4 protein surface.

| Aminoacid Residues | C-4 | C-5 | C-6 |
|--------------------|-----|-----|-----|
| Exametane          | Trp5 | Trp5 | Trp5 |
| Cys94              | Asn62 | Asn62 | Asn62 |
| His96              | His64 | His64 | His64 |
| His119             | Glu67 | Glu67 | Glu67 |
| Val121             | Gln69 | Gln69 | Gln69 |
| Val142             | Ile70 | Ile70 | Ile70 |
| Leu197             | Gln72 | Gln72 | Gln72 |
| Thr198             | His74 | His74 | His74 |
| Thr199             | Val212 | Val212 | Val212 |
| Val206             | Phe213 | Phe213 | Phe213 |
| Trp208             | Leu214 | Leu214 | Leu214 |

Table 5. Distance involved in the interaction between compounds 4 to 6 with 3HB4 protein surface.

| Comp. | Ketone (Å) | Cyanide (Å) | Imino (Å) | Indole (Å) | Hydroxyl (Å) | Ester (Å) |
|-------|------------|-------------|-----------|------------|--------------|-----------|
| 4     | Ser19 (13.98) | -           | Ty113 (11.90) | Cys185 (17.79) | Cys185 (20.69) | Let94 (14.10) | Ser19 (19.89) | Leu96 (14.74) | Ser19 (13.37) | - |
| 5     | Ser19 (15.44) | Ty113 (15.14) | Cys185 (20.51) | Ser19 (25.90) | Ser19 (19.89) | Leu96 (13.31) | Ser19 (10.66) | Asn52 (2.72) | Ser19 (5.58) | Cys185 (15.32) |
| 6     | Ser19 (22.27) | Leu96 (16.88) | Ser19 (11.53) | Ty113 (11.53) | Cys185 (7.65) | Phe107 (16.19) | Ser19 (25.90) | Ser19 (19.89) | Leu96 (13.31) | Ser19 (10.66) | Asn52 (2.72) | Ser19 (5.58) | Cys185 (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 determined using DockigServer [27]. Theoretical data (Table 6 and 7) indicate that there are differences in the thermodynamic factors values of exematane and fisetin compared with compounds 4 to 6.

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

| Compound | Energy of Binding (kcal/mol) | ΔHbnd | ΔSolv | ΔGbind | ΔElectro | ΔIntermolec | ΔInteract. | Surface |
|----------|-----------------------------|--------|--------|--------|----------|-------------|-----------|---------|
| Exematan | -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 exematane 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 2WD3 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 3BH4 protein surface.

| Compound | Est. Fee Energy of Binding (kcal/mol) | Est. Inhibition Constant, Ki (µM) | edw + Hbnd + desolv Energy | Electrostatic Energy | Total Intermolec. Energy | ΔInteract. | 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        | -11.90                                 | 3.15                              | -12.56                    | -0.07               | -12.83                   | 1094.61   |         |
| 6        | -2.90                                  | 7.47                              | -3.23                     | -0.07               | -3.30                    | 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 a 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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