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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**).

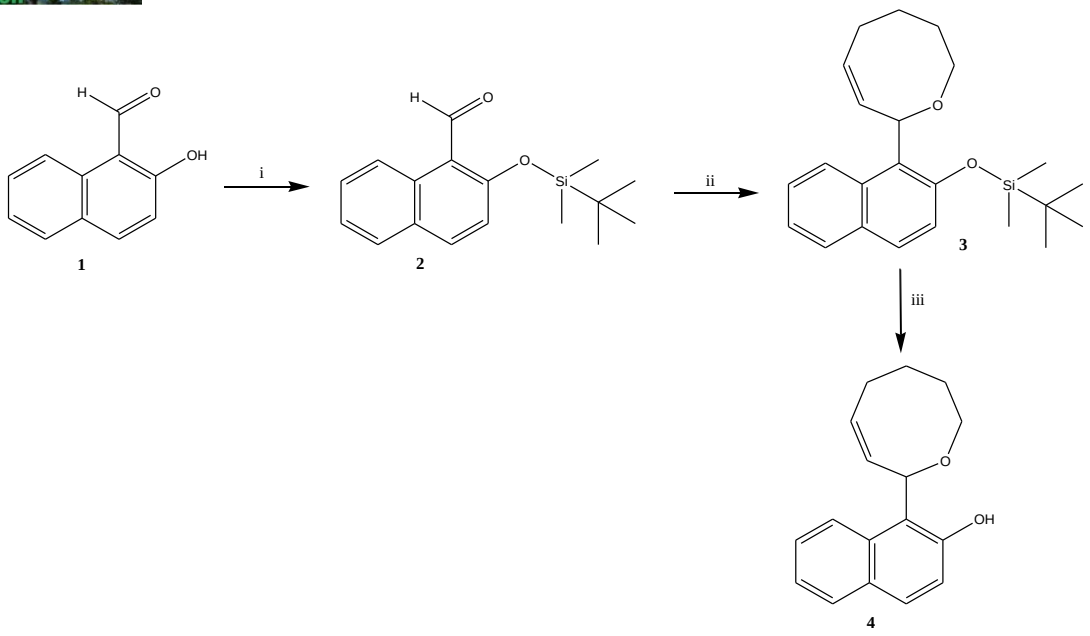

Figure 1. Synthesis of an oxocin-naphthalen-2-ol derivative (**4**). Reaction of 2-hydroxy-1-naphthaldehyde (**1**) with terbuthyldimethyl-sylane chloride (i) to form the 2-(tert-Butyl-dimethyl-silanyloxy)-naphthalene-1-carbaldehyde (**2**). Then **2** was reacted with 5-hexyn-1-ol (ii) to synthesis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

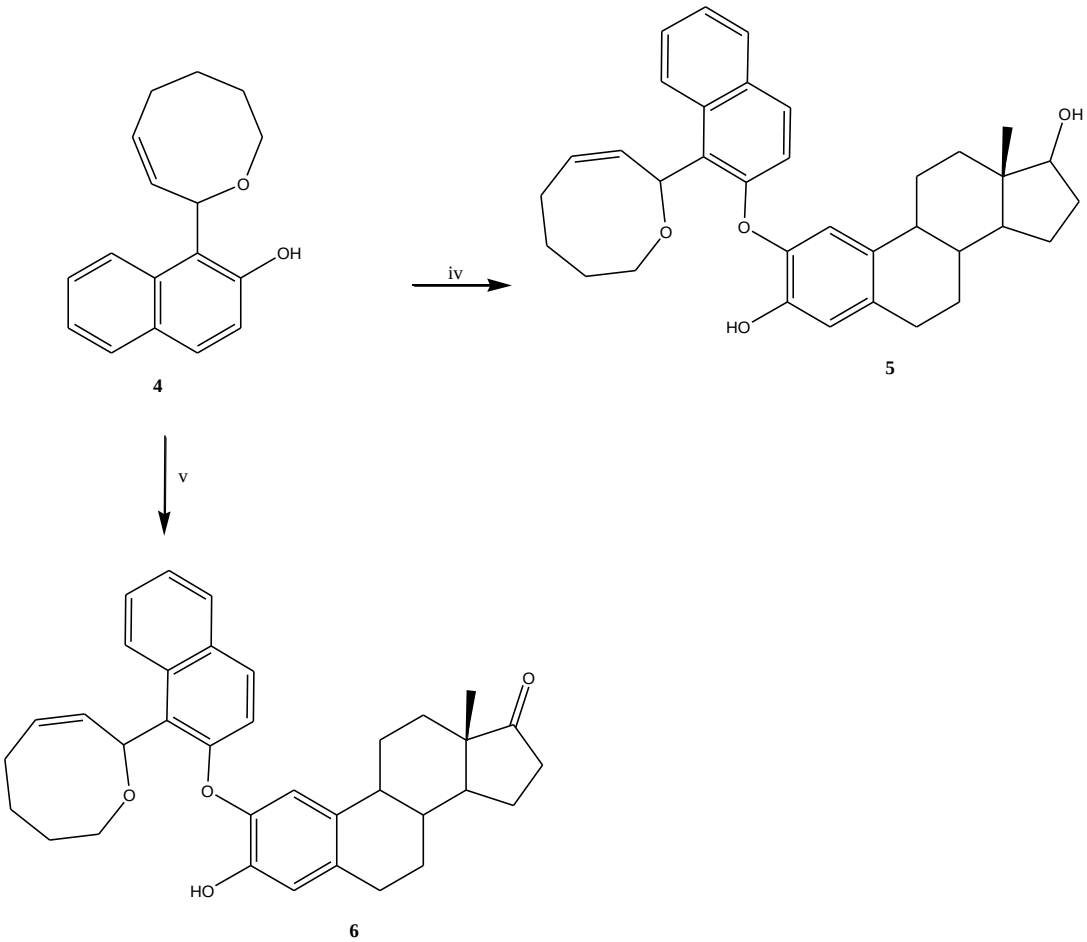

Figure 2. Preparation of two oxin-steroid derivatives (**5** or **6**). Reaction of oxocin-naphthalen-2-ol derivative (**4**) with 2-nitroestradiol (iv) or 2-nitroestrone (v) to form the oxin-naphalen-cyclopenta[a]phenanthrene-1,7-diol (**5**) or oxin-naphalen-cyclopenta[a]phenanthrene-1-one (**6**).

^{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.

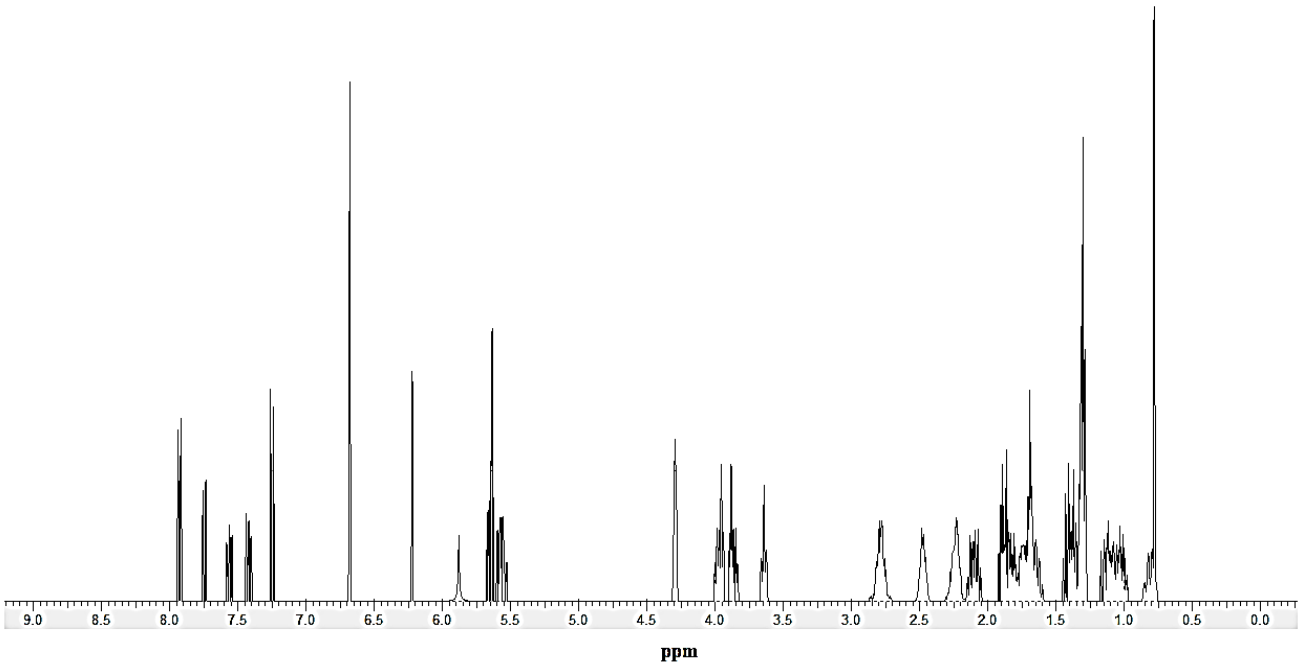

Figure 3. ^{1}H NMR spectrum of **5** was determinate with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in $CDCl_3$. ppm = parts per million.

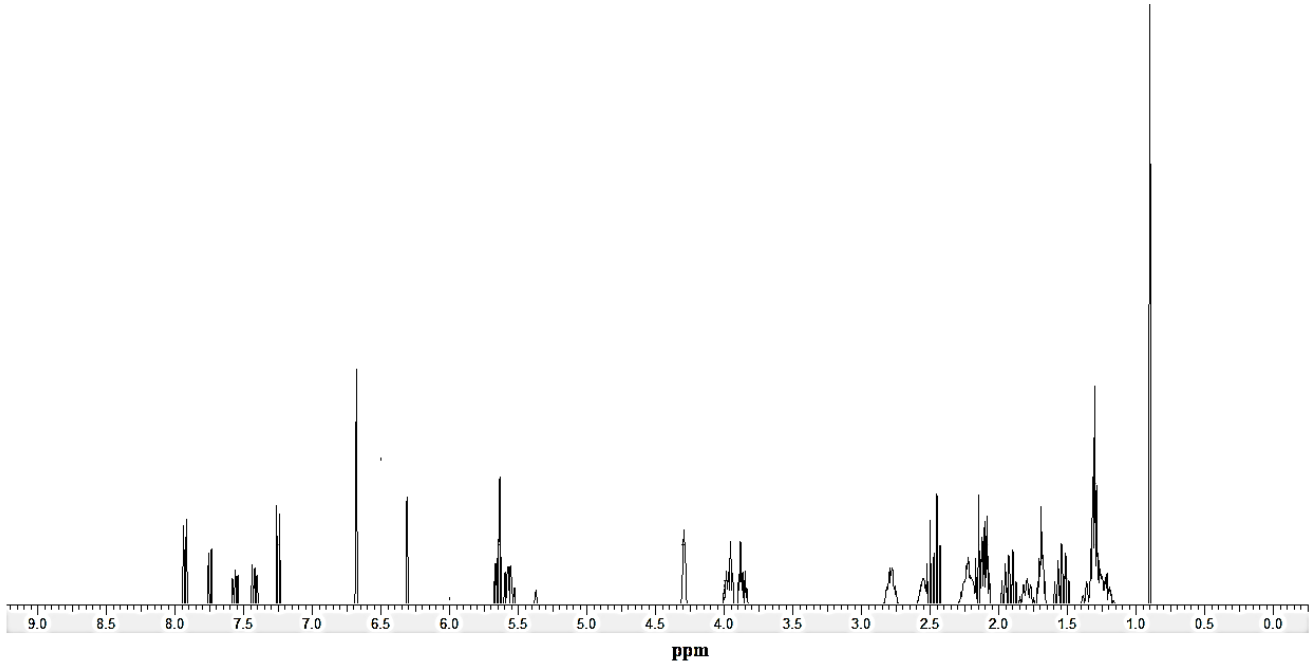

Figure 4. The scheme shown ^{1}H NMR spectrum of compound **6**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 and 75.4 MHz in $CDCl_3$. ppm = parts per million.

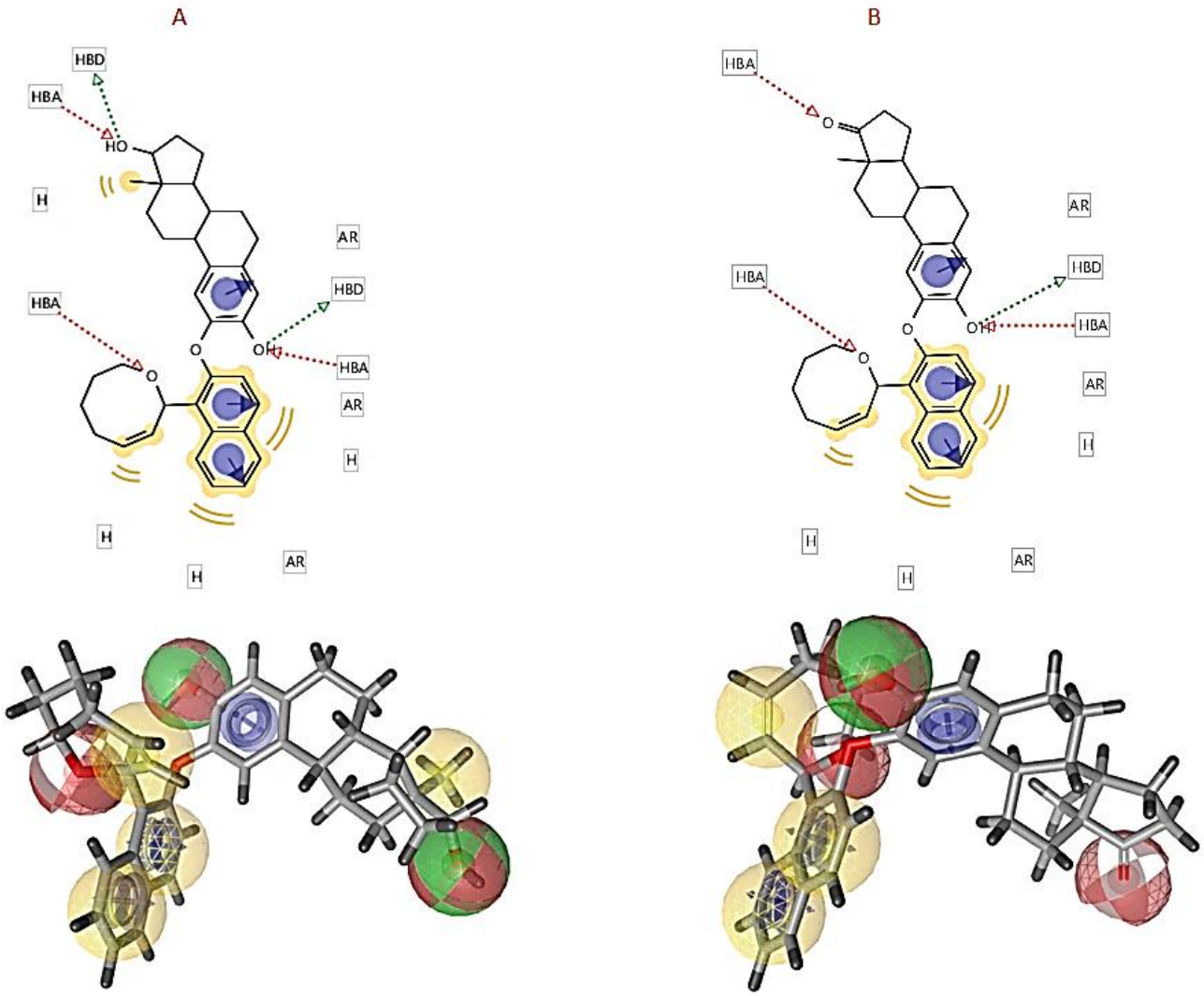

Figure 5. Scheme represents a pharmacophore from both compounds **5** (A) and **6** (B) using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

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).

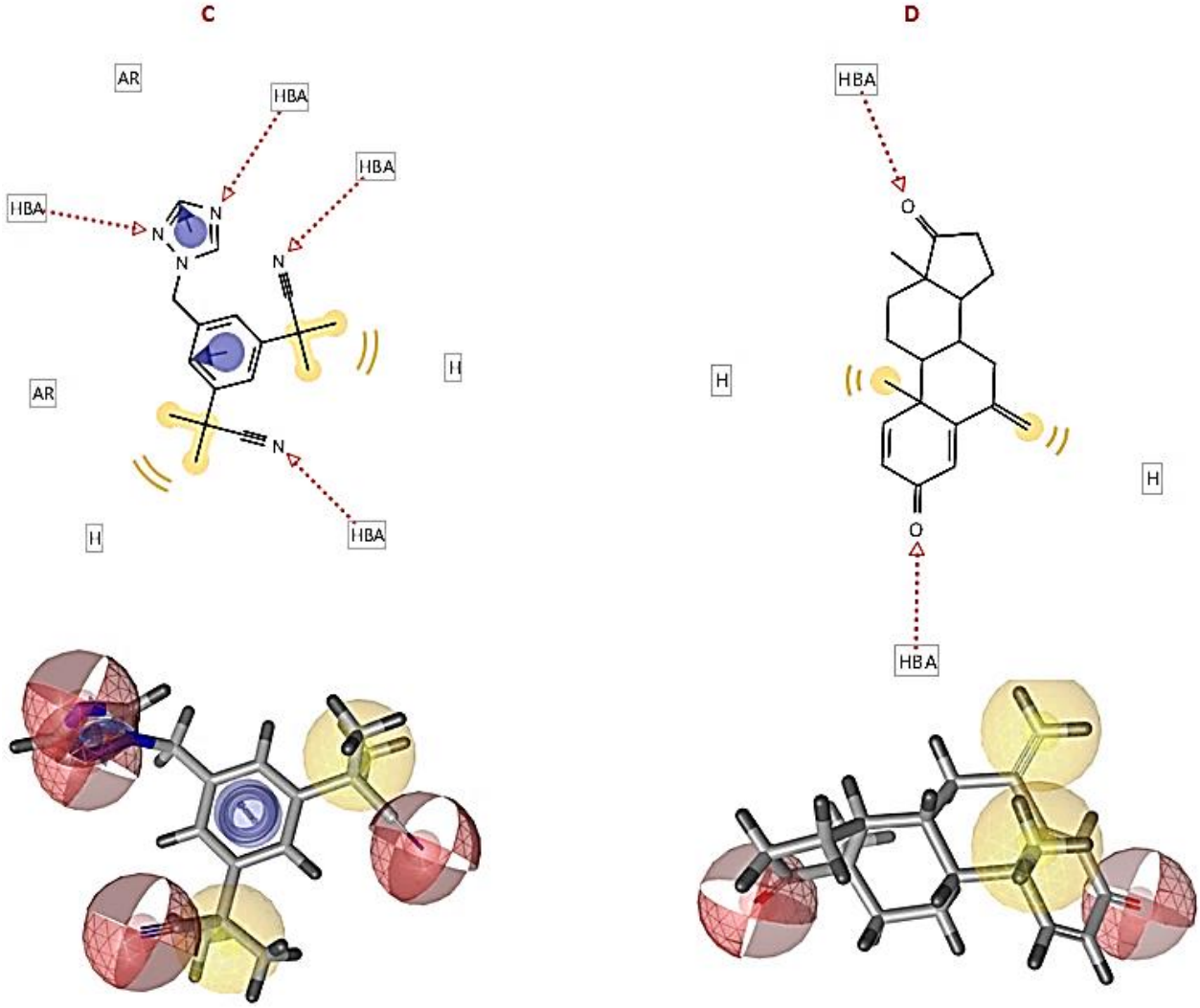

Figure 6. Pharmacophore from both anastrozole (C) and exemestane (D) using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red), hydrogen bond donor (HBD, green) and a positive ionizable (PI).

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.

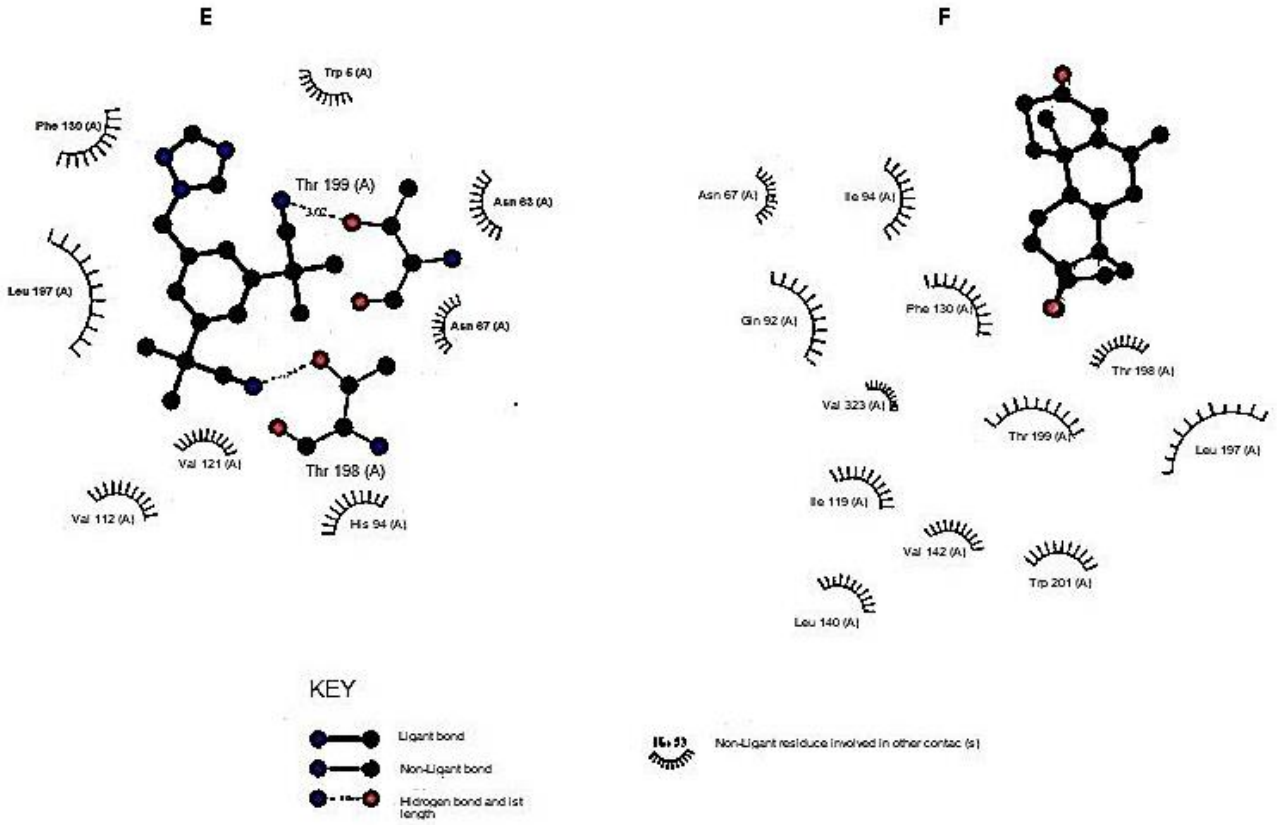

Figure 7. The scheme shows the binding of both anastrozole (E) and exemestane (F) with some aminoacid residues of the aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

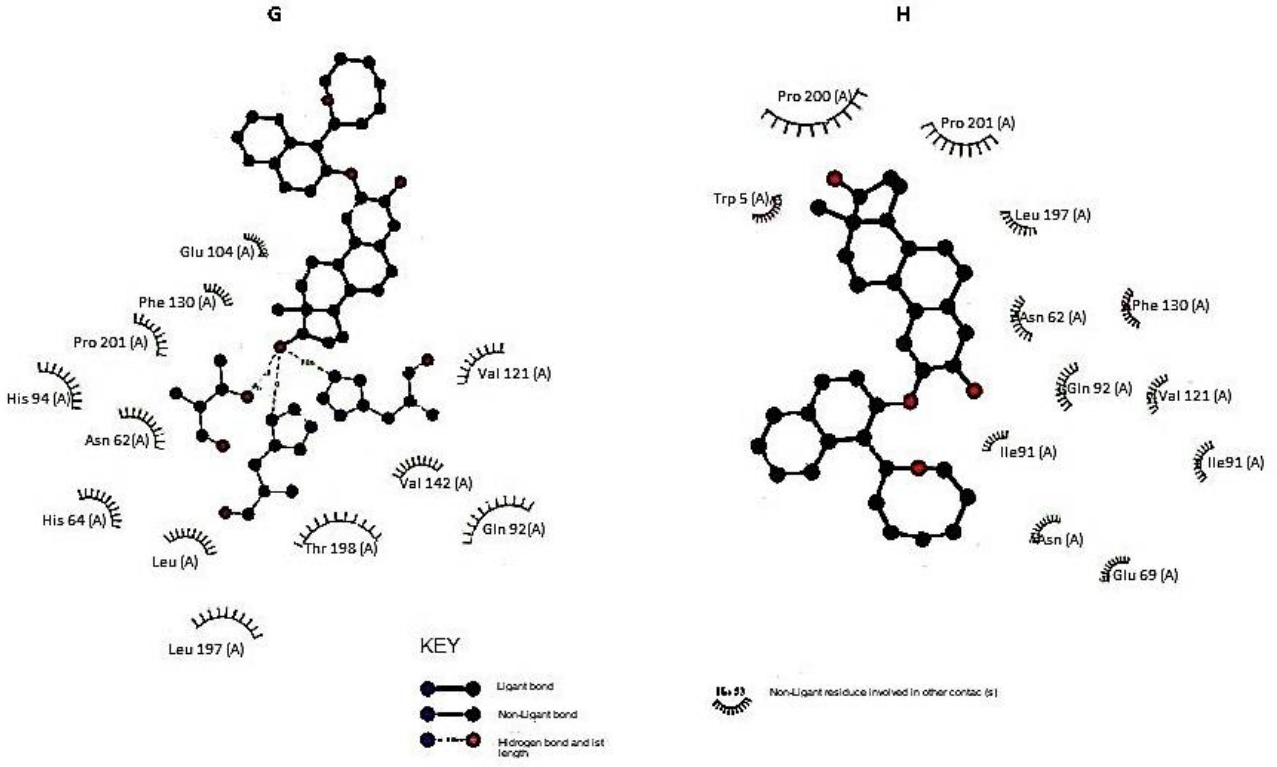

Figure 8. The scheme shows the binding of both compounds **5** (G) and **6** (H) with some aminoacid residues of the aromatase enzyme (4kq8). The visualization was carried out with Dockingserver software.

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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