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Molecular Docking Studies of Estrone-Coumarin Derivatives as Aromatase and 17β-HSD1 Inhibitors Related to Hormone Receptor Positive (HR+) Breast Cancer

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Abstract

Hormone Receptor positive (HR+) breast cancer is the most common malignancy in women. New strategies in the treatments have targeted the estrogen biosynthesis pathways including the inhibition of the aromatase and 17β-HSD1 enzymes. The present work, describes the study of a new family of 9 hybrid compounds derived from estrone attached to a coumarin fragment, linked through different lengths of hydrocarbon chains. The activity of these compounds was evaluated by molecular docking with two relevant enzymes in breast cancer (HR+). It has been proposed nine compounds as 17β-HSD1 inhibitors and six as aromatase inhibitors. We found important interactions with key amino acids at the orthosteric site of each enzyme and their score values compared to the crystallographic ligand. The *in silico* analysis showed good score values in the proposed compounds, where the steroidal portion presented important interactions with Met374 and Tyr155 in aromatase and in 17β-HSD1 respectively. Highlighting Compounds **2**, **5** and **8** with an aromatic ring at the C4 position of the coumarin moiety, which favored arene-H type interactions essential for protein-ligand recognition. In addition, the results related to the 17β-HSD1 enzyme demonstrated how the length of the linker influences the interaction; the best score was found for derivative **8** with a chain of 8 methylenes.

Keywords

Estrone, Coumarins, Aromatase Inhibitors, 17β-HSD1 Inhibitors, Molecular Docking

1. Introduction

Hormone Receptor positive (HR+) breast cancer is the most aggressive and frequent malignant neoplasm in women, representing the main cause of oncological mortality [1]. In this regard, aromatase inhibitors are compounds that suppress estrogen biosynthesis through reversible blockade of this enzyme and are mainly used as aids in the treatment of this condition [2]. Aromatase belongs to the group of the cytochrome P450 family, and in breast tissue, it catalyzes the demethylation of carbon 19 of androstenedione, producing an 18-carbon phenolic estrogen, estrone E1 [3]. Furthermore, estrone sulfate is the most abundant circulating estrogen in postmenopausal women and it can be converted to estrone via sulfatase [4]. In the same way, the 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) enzyme is involved in the catalytic reduction of estrone to estradiol in both pathways and it is overexpressed in breast cancer cells [5]. Moreover, the inhibition of this enzyme prevents the formation of estradiol (E2), a hormone that stimulates the growth of breast tumors and endometriosis through the activation of the Estrogen Receptor α (ERα) [6], therefore, it was proposed as an interesting pharmacological target.

It has been described steroidal and non-steroidal inhibitors for these molecular targets, steroid inhibitors usually bind reversibly to the catalytic site of the enzyme, mimicking either the substrate or the product. In this context, aromatase and 17β-HSD1 enzymes have in common that estrone bind to both [7].

However, these antiestrogen compounds have limitations: their mixed agonist, antagonist profile (ERα), and the development of tumor resistance, while the non-steroidal inhibitors showed low selectivity and inhibits other CYP450 isoforms. Based on the above, it is evident the necessity of new molecules with dual action, the selective inhibition of both targets and simultaneously to reduce the risk of adverse effects, drug interactions and resistance (**Figure 1**) [8]-[14].

Coumarins are molecules formed by the union of a benzene and an α-pyrone [15]. These molecules have shown a wide variety of pharmacological activities, mainly antiproliferative effects. In addition, it has been observed that coumarins show *in vitro* inhibitory activities of some enzymes involved in cancer, which could secondarily attenuate the clinical progress and the pathophysiological manifestations observed in this disease [16] [17] [18] [19]. Moreover, estrone derivatives or other steroidal compounds have greater specificity than non-steroidal ones for binding to enzymes involved in estrogen biosynthesis [20] [21]. Considering the described above, we chose a model of study derivatives of estrone bearing a coumarin fragment.

Leonetti *et al.* have reported that the chain length of carbon atoms that function as linkers, directly influences the potency of the molecules. The absence or the presence of very short or very large linkers results in the decrease of aromatase inhibition and selectivity (molecular promiscuity). Therefore, the length of the linker can affect the inhibitory potency by changing the position of the H-bond acceptors in the ligand binding site [22] [23] [24].

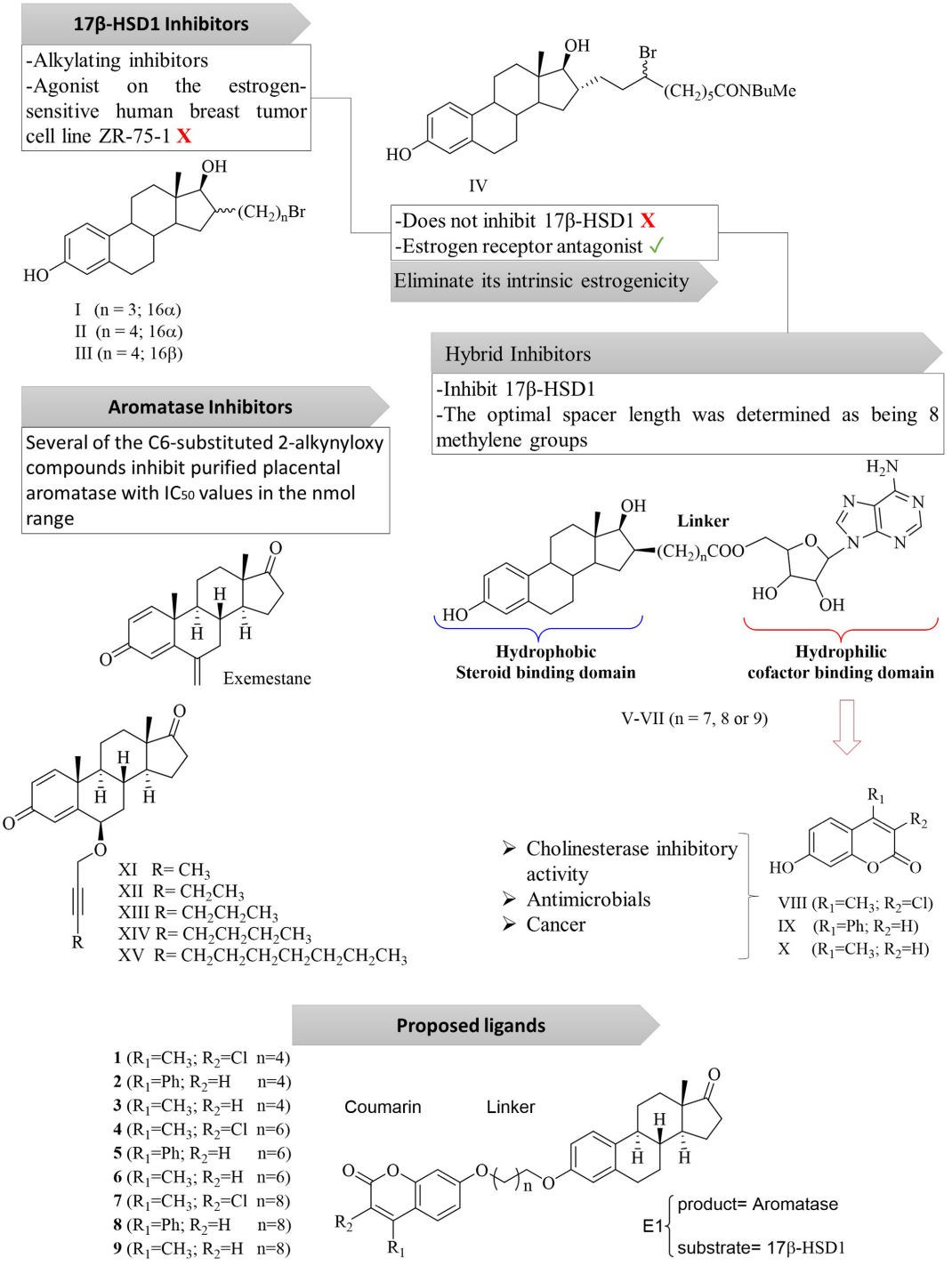

Figure 1. Known aromatase and 17β-HSD1 inhibitors and proposed Compounds **1 - 6**.

The drug development process requires a multidisciplinary effort comprising main stages: 1) target selection and validation; 2) compound screening and lead optimization; 3) preclinical studies; and 4) clinical trials [25]. Therefore, this whole process takes an average of 800 million dollars and 15 years to complete [26]; for this reason, tools such as Computer-Aided Drug Design (CADD) have gained popularity in recent years, playing an important role in drug develop-

ment. Automated Molecular Docking is the most widely used instrument in CADD, its purpose is to propose a binding mode between two molecules, with overall minimum energy, considering the principle of complementarity of the structures and interaction forces involved in molecular recognition [27] [28]. In this sense, there are different types of interactions that can be essential in the affinity of a ligand with a protein target and in the stability of a ligand-protein complex. The nature of the interactions is diverse, for example: hydrogen-bridging type interactions (caused by steric effects due to entropy and solvent-related forces), electrostatic forces (charges), van der Waals interactions (electrodynamics forces) and hydrophobic interactions. The type of interaction observed is dependent on the size, shape, and physicochemical properties of the amino acid residues of the binding pocket and the class and flexibility of the ligands [29] [30].

Thus, Molecular Docking is very useful to predict the binding mode of a ligand to a protein and its possible affinity to it, optimizing the development and discovery of new bioactive molecules and/or drugs. It also improves the designation of the leading compounds through the structure-activity relationship, and facing the synthesis stage, it helps to filter and prioritize certain compounds from a large collection of molecules, saving time and costs.

Accordingly, it was decided to carry out molecular docking only of Compounds (**1 - 6**) with linkers of 4 and 6 carbon atoms on the aromatase enzyme.

The proposed molecules **1 - 6** (**Figure 1**) were designed based on the following pharmacophoric fragments: three coumarins as a polar group (non-steroidal fragment) attached through hydrocarbon chains of different lengths to estrone as an enzyme recognition group. In this work, we have designed and screened *in silico* a series of new estrone derivatives with different groups of coumarins against aromatase and 17β-HSD1 enzymes to find new chemical entities that could achieve benefits in the treatment of breast cancer (HR+).

2. Materials and Methods

2.1. Molecular Docking

The crystal structures of the aromatase and 17β-HSD1 were obtained from the Protein Data Bank (https://www.rcsb.org/) with the codes PDB: 5 JKW and 1 FDT, respectively. The molecular docking was carried out with the Molecular Operating Environment software (https://www.chemcomp.com/MOE%20v2020.0901).

The unnecessary molecules were removed (H_2O and SO_4), the hydrogen atoms, charges and energy minimization were adjusted with the AMBER10: EHT force field and R-field solvation from the MOE suite. The ligands (**1 - 6**) were built with ChemDraw Professional 16.0 software. The minimization and ionization of the compounds were performed with AMBER10: EHT as the force field. As a placement function Triangle matcher was selected, the scores were calculated with the London ΔG function and the selection of the best poses was made using the GBVI/WSA ΔG (Generalized-Born Volume Integral/Weighted Surface area)

scoring function. These parameters were established for docking validation. The binding site was defined using the crystallographic ligand (orthosteric site). For each ligand it was generated 100 repetitions and carried out 100 poses. The best binding poses were visually inspected, the pose that most overlapped with the crystallographic ligand was chosen and its scores (ΔG = kcal/mol) was reported. The graphical representations of ligand interactions were created in MOE.

2.2. Docking Validation

The Docking protocols were validated, by a redocking into the aromatase and 17β-HSD1 ligand-binding sites with its co-crystal-ligands Testosterone (TST) and estradiol (E2) respectively. The Root Mean Square Deviation (RMSD) between the co-crystal ligand and the redocked molecule of aromatase was 0.2912 ± 0.018 Å and to the17β-HSD1 was 0.1381 ± 0.0086 Å. Validation was performed in triplicate.

3. Results and Discussion

3.1. Aromatase (CYP19A1)

To validate our model preparation, the Root Mean Square Deviation (RMSD) was included as a measure of re-docked success. In which a value of 0.2912 ± 0.018 Å was obtained. **Figure 2** shows the pose with the lowest RMSD and a score of −8.3711 kcal/mol. It can be observed that the hydrophobic side chains Trp224, Phe221, Phe134, Ile133, Val370 and Met374 are placed around the testosterone molecule (TST, crystallographic ligand), shaping the ligand binding pocket (**Figure 2**). In addition, TST bonds with its 17β-hydroxyl oxygen and receives a hydrogen bond from the amide backbone -NH of Met374 [31] [32] [33].

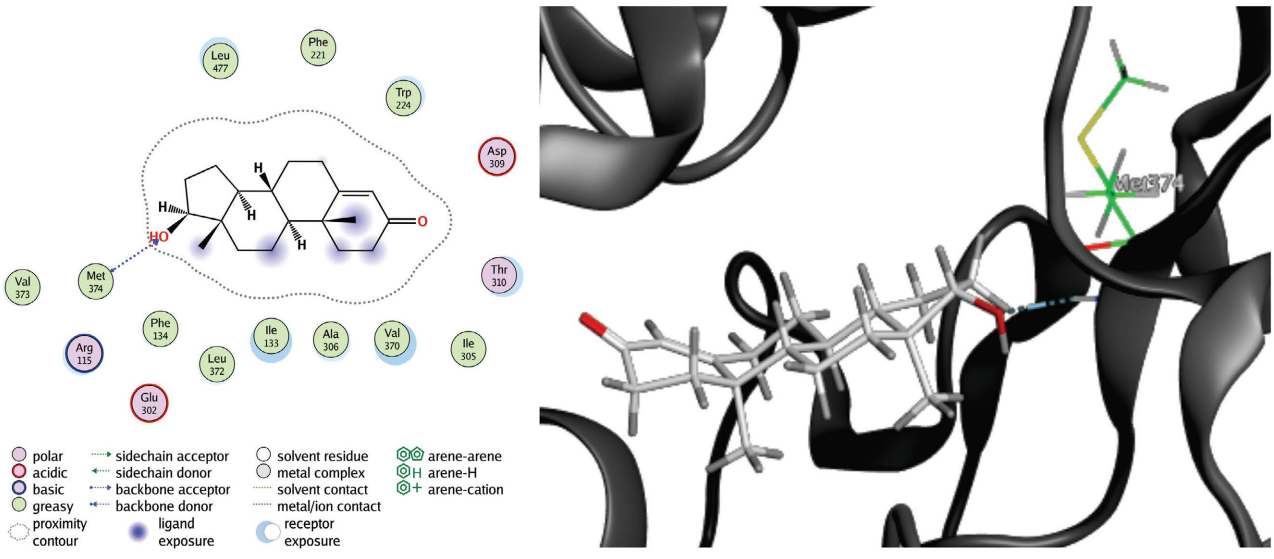

Figure 2. 2D interaction diagrams and its three-dimensional pose of the TST in the binding pocket of the aromatase enzyme (5 JKW).

To explain the presumable binding mode with human aromatase, Compounds **1 - 6** were subjected to an *in silico* molecular docking. The results revealed that Compounds **1 - 6** fit into the binding pocket and the carbonyl group (C17) of the steroid moiety form a hydrogen bond interaction with Met374. This portion achieved good overlap with the TST at the binding site and exhibited hydrophobic interactions with the amino acids that make up this region (**Figure 2**). It is important to mention that Compounds **2** and **5** obtained the best scores of −12.0826 kcal/mol and −12.0233 kcal/mol, respectively (**Figure 3**). It is known that aromatic rings can act as hydrogen bond acceptors stablishing meaningful interactions with the amino acid residues [34], the benzene ring attached to the coumarin fragment in Compounds **2** and **5** forms a H-arene interaction type with the amino acids Val369 and Val370.

Unlike most cytochrome P450 enzymes, near the active site of the aromatase there is a cavity large enough to shelter a secondary interaction with the substrate or with an additional non-steroidal molecule, this interaction could influence the enzymatic activity of the aromatase [22]. In this context, the coumarin

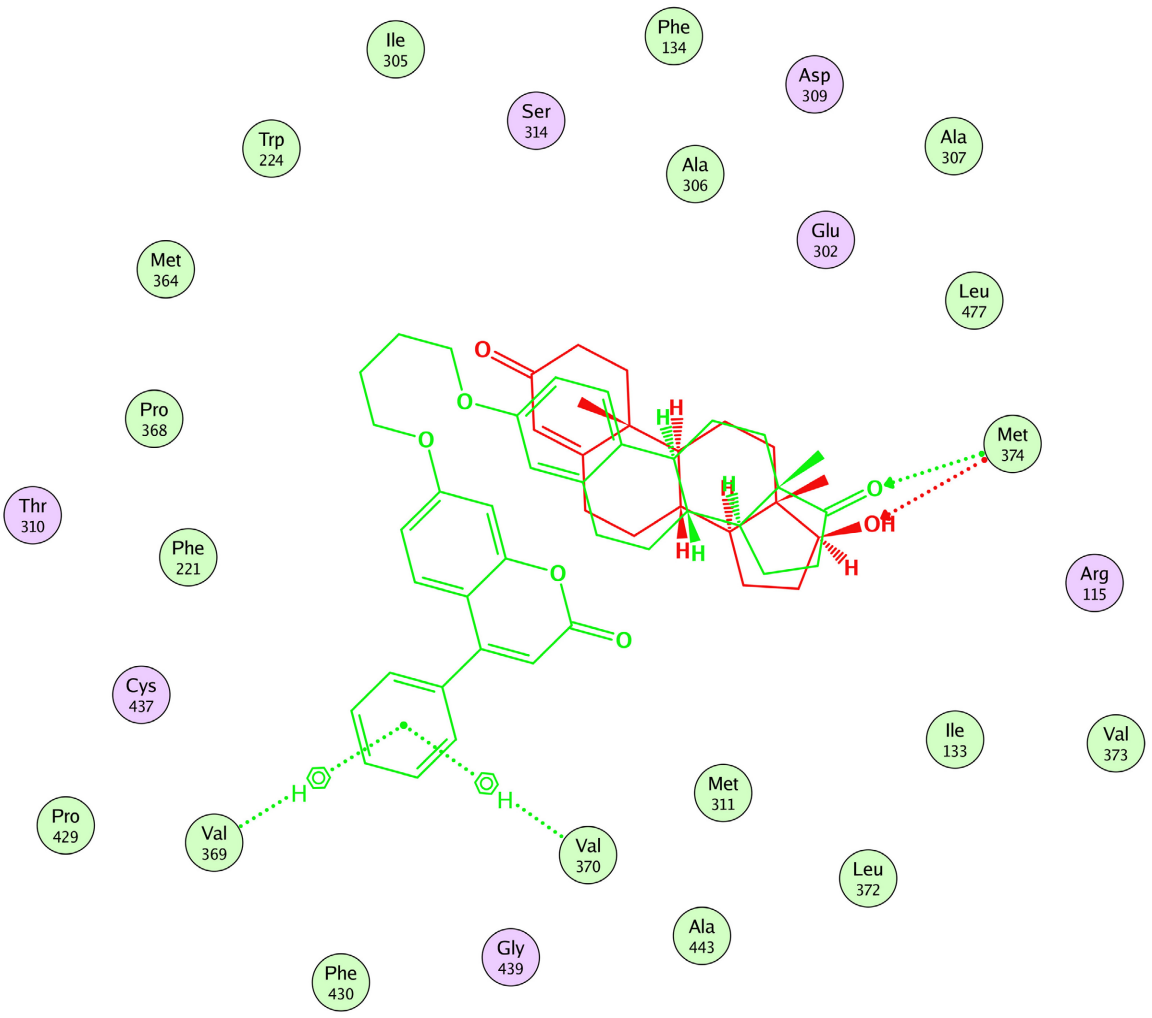

Figure 3. 2D overlay of the crystallographic ligand TST (red) and docked pose of Compound **2** (green).

moiety of the studied compounds showed interactions involving the aromatic rings, these interactions are critical for protein-ligand recognition [35]. Compounds (**2** and **5**) substituted with a benzene ring at C4 of the coumarin portion (**Figure 4**), showed arene-H type interactions, the electron-rich π cloud interacts with hydrogen atoms (Δ+) of the amino acids Val369 and Val370 in Compound **2** (-NH), and with Ala438 in Compound **5** (-CH_3). These additional interactions could be related with the score values obtained by the different ligands (**Table 1**), and therefore, with their probably affinity to the aromatase.

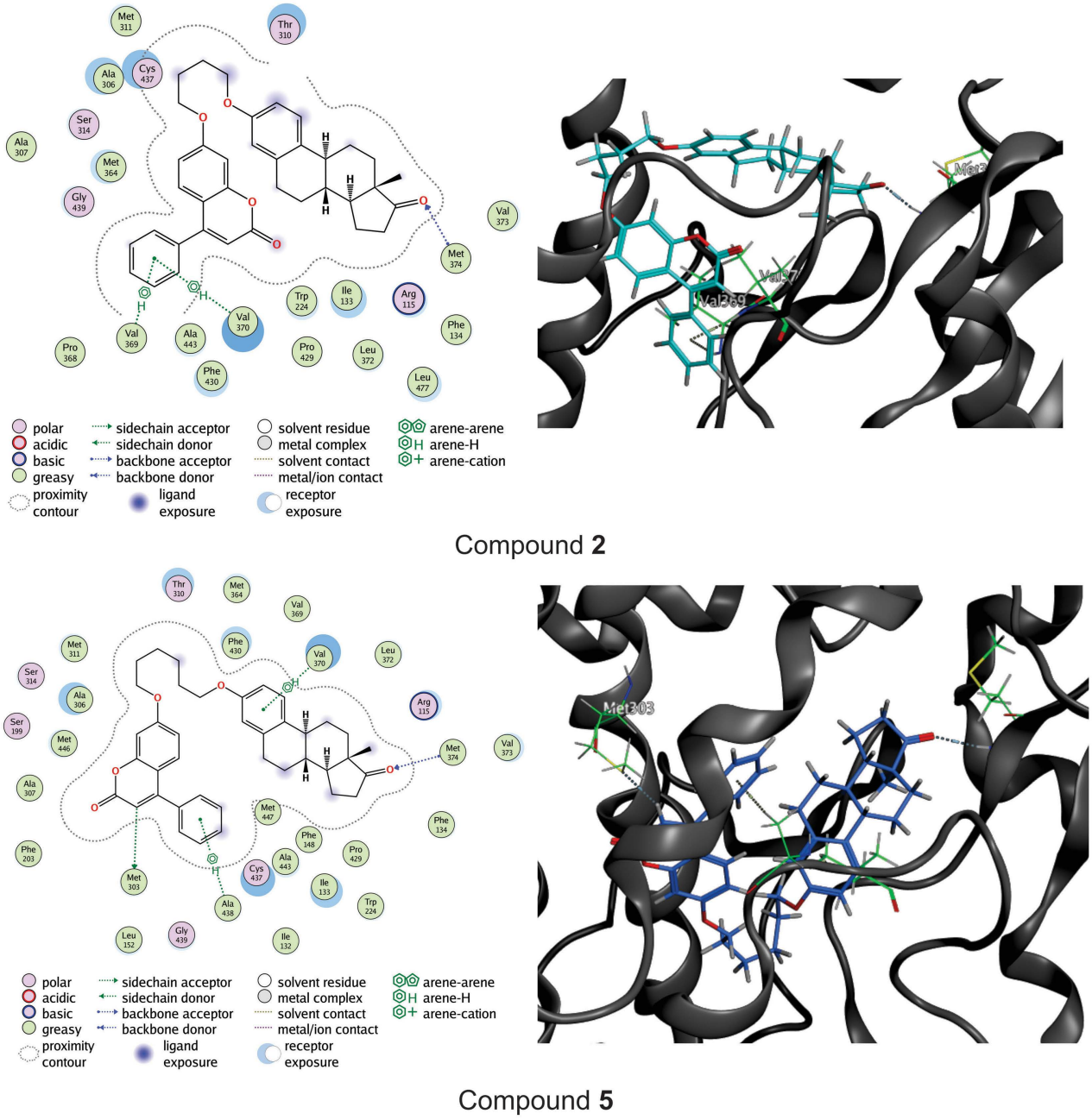

Figure 4. 2D interaction diagrams and its three-dimensional poses of Compounds **2** (ΔG = −12.0826 kcal/mol) and **5** (ΔG = −12.0233 kcal/mol) in the aromatase crystal structure obtained with the MOE program.

Table 1. Additional interactions with the aromatase enzyme.

Ligand	ΔG Binding	Conventional Hidrogen Bond	Hydrophobic Interactions	Others
*TST	−8.3711	Met374	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477	
1	−11.3929	Met374	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477 Ala307 Met311 Ala443 Phe430 Val369 Pro429 Met364 Ile132 Leu152 Met303 Ala438 Met446 Phe148 Ile442	Gly439
2	−12.0826	Met374	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477 Ala307 Met311 Ala443 Phe430 Val369 Pro429 Met364 Pro368	Val369 Val370
3	−11.3581	Met374 Ala438	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477 Ala307 Met311 Ala443 Ile132 Leu152 Met303 Ala438 Met446 Phe148 Ile442	
4	−11.7687	Met374	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477 Ala307 Met311 Ala443 Phe430 Pro429 Met364 Leu152 Met303 Ala438 Met446 Met447	
5	−12.0233	Met374	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477 Ala307 Met311 Ala443 Phe430 Val369 Pro429 Met364Ile132 Leu152 Met303 Ala438 Met446 Phe148 Met447 Phe203	Met303 Val370 Ala438
6	−11.7957	Met374	Trp224 Phe221 Phe134 Ile133 Val370 Met374 Ile305 Ala306 Val373 Leu372 Leu477 Ala307 Met311 Ala443Ile132 Leu152 Met303 Ala438 Met446 Phe148 Ile442	Met303 Cys437

Moreover, Compounds **1**, **3** and **6** displayed hydrogen bond interactions with Met374, a key residue for the ligand recognition and aromatase inhibition; furthermore, they showed additional interactions at the coumarin fragment. Compound **1** showed π-H type interactions with the Gly439 residue and the *α*-pyrone ring of the coumarin skeleton, Compound **3** formed a hydrogen bond with Ala438, residue which acts as a backbone donor, while for Compound **6**, the residues Met303 and Cys437 (amino acids with a sulfur atom), behaved as side chain acceptors (Figure 5).

According to the structure/score relationship analysis (binding energy) it was predicted for Compounds **1 - 6** better scores than those of the crystallographic ligand TST. Moreover, no relevant difference was observed in the binding energy between the compounds with a chain of 4 and 6 carbon atoms (Figure 6). However, Compounds **2** and **5** with the benzene substituent at position 4 of the coumarin fragment obtained the best scores of −12.0826 and −12.0233 kcal/mol, respectively. This observation indicates that the aromatic type substituents improve and even reinforce the interaction with the aromatase binding site.

3.2. 17*β*-Hydroxysteroid Dehydrogenase Type 1 (17*β*-HSD1)

In the validation of the 17*β*-HSD1 enzyme, an RMSD value of 0.2067 ± 0.0228 Å was obtained. Figure 7 shows the pose with the lowest RMSD and a score of

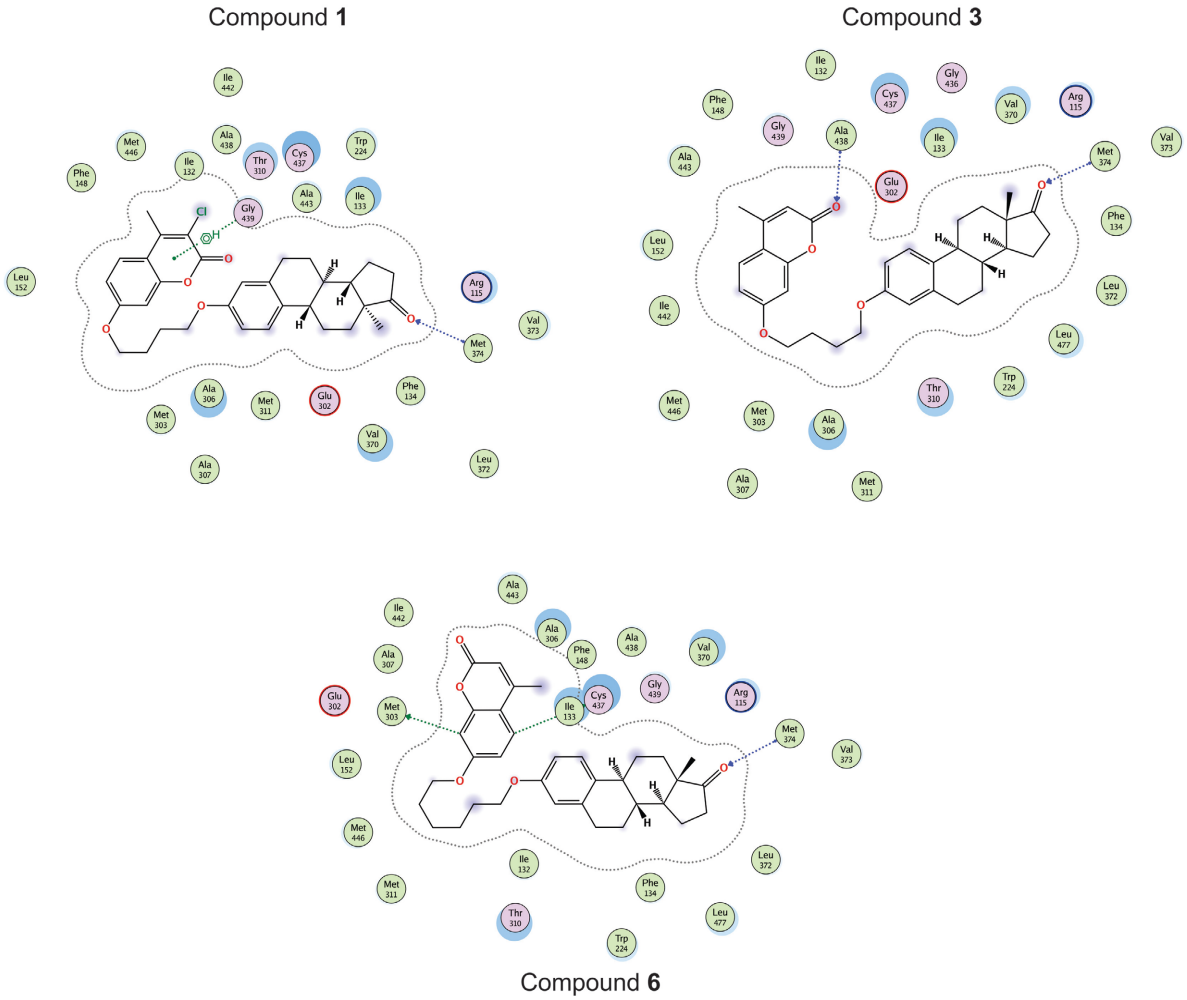

Figure 5. 2D interaction diagrams of Compounds **1** (ΔG = −11.3929 kcal/mol), **3** (ΔG = −11.3581 kcal/mol) and **6** (ΔG = −11.7957 kcal/mol) in the aromatase crystal structure obtained with the MOE program.

−7.2788 kcal/mol. In this image, estradiol (E2, crystallographic ligand) is located near the catalytic triad (Ser142, Tyr155 and Lys195), the 17β-hydroxyl oxygen accepts a hydrogen bond from the -OH group of Tyr155.

The lipophilic pocket of the 17β-HSD1 is formed by Gly92, Leu93, Gly94, Ala291, Phe192 and Val196, which is connected to a hydrophilic zone composed of Ser142, Tyr155, Glu194 and Lys195; this hydrophilic zone is crucial for the catalytic reaction of estradiol [36] [37]. It has been observed that some inhibitors of 17β-HSD1, with a steroidal moiety attached to a polar fragment through different methylene chains, establish interactions between the polar group and the cofactor binding site. Among the compounds tested, those with the best results are the derivatives with linkers of 6 or 8 methylenes. [11] [38] [39] [40].

To favor secondary interactions that might improve the scores observed for 17β-HSD1, it was added three compounds bearing eight carbon atoms as a linker. The nine Compounds **1** - **9** showed interactions of the arene-H type with Tyr155 (**Figure 8** and **Figure 9**) and some of them with the hydrophobic residues Pro187 and Phe259 at the binding site.

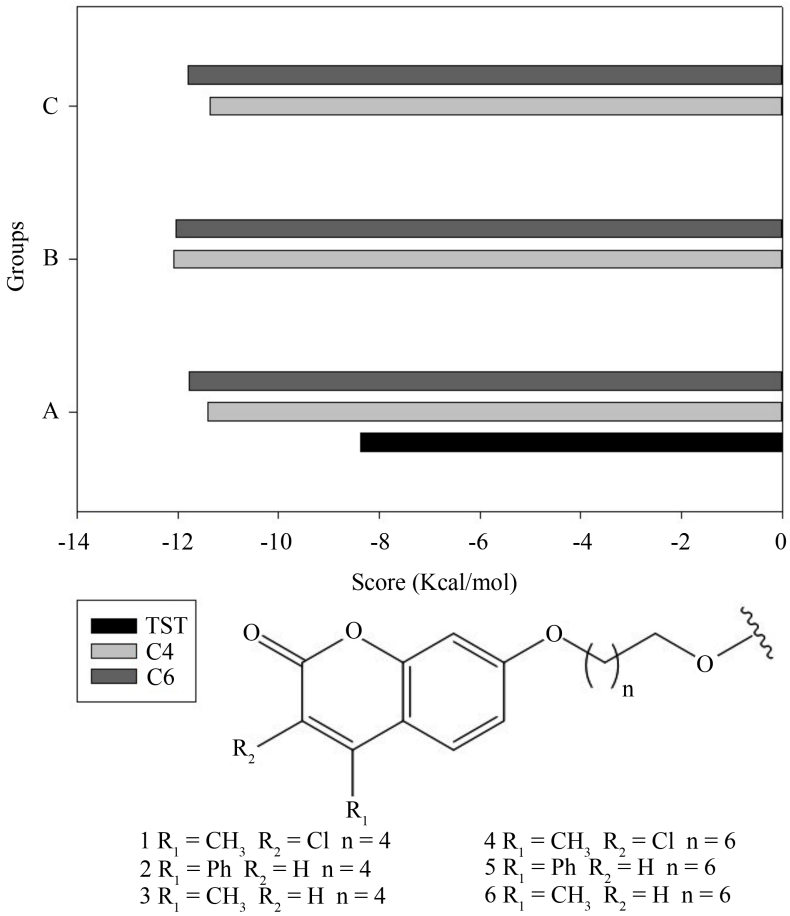

Figure 6. Effect of string length on score values. Group A: Compounds **1** and **4**; Group B: Compounds **2** and **5**; Group C: Compounds **3** and **6**.

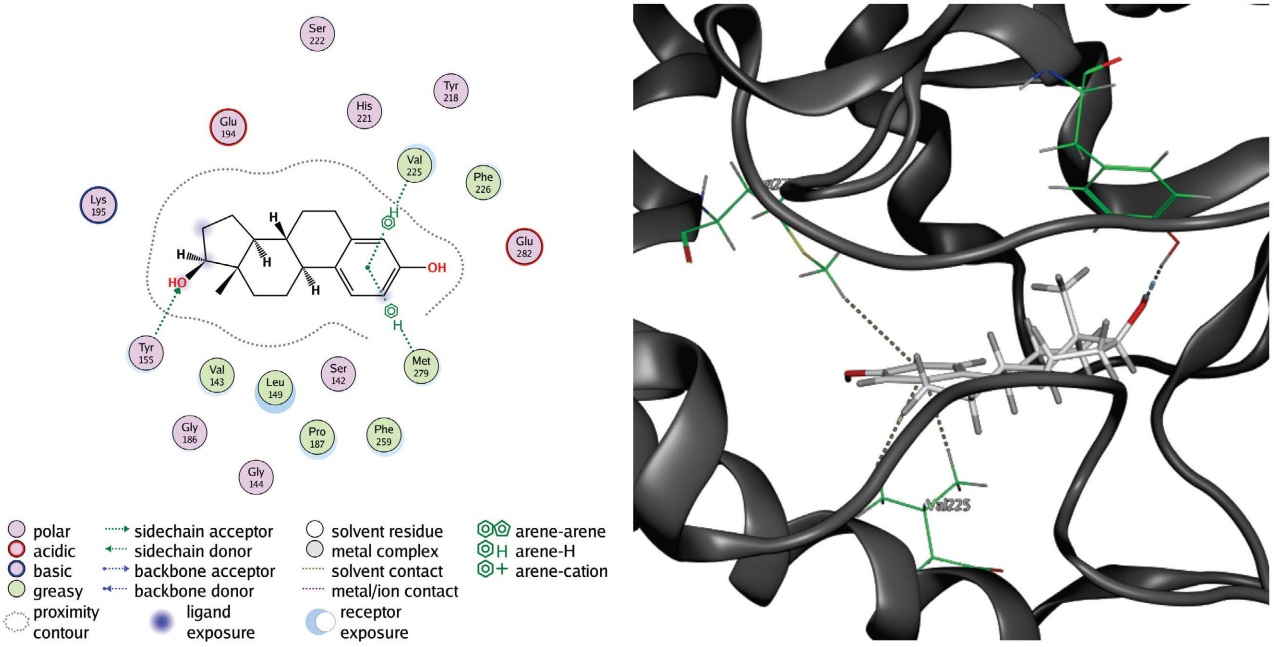

Figure 7. 2D interaction diagram and its 3D pose of E2 in the binding pocket of the 17β-HSD1 enzyme (1FDT).

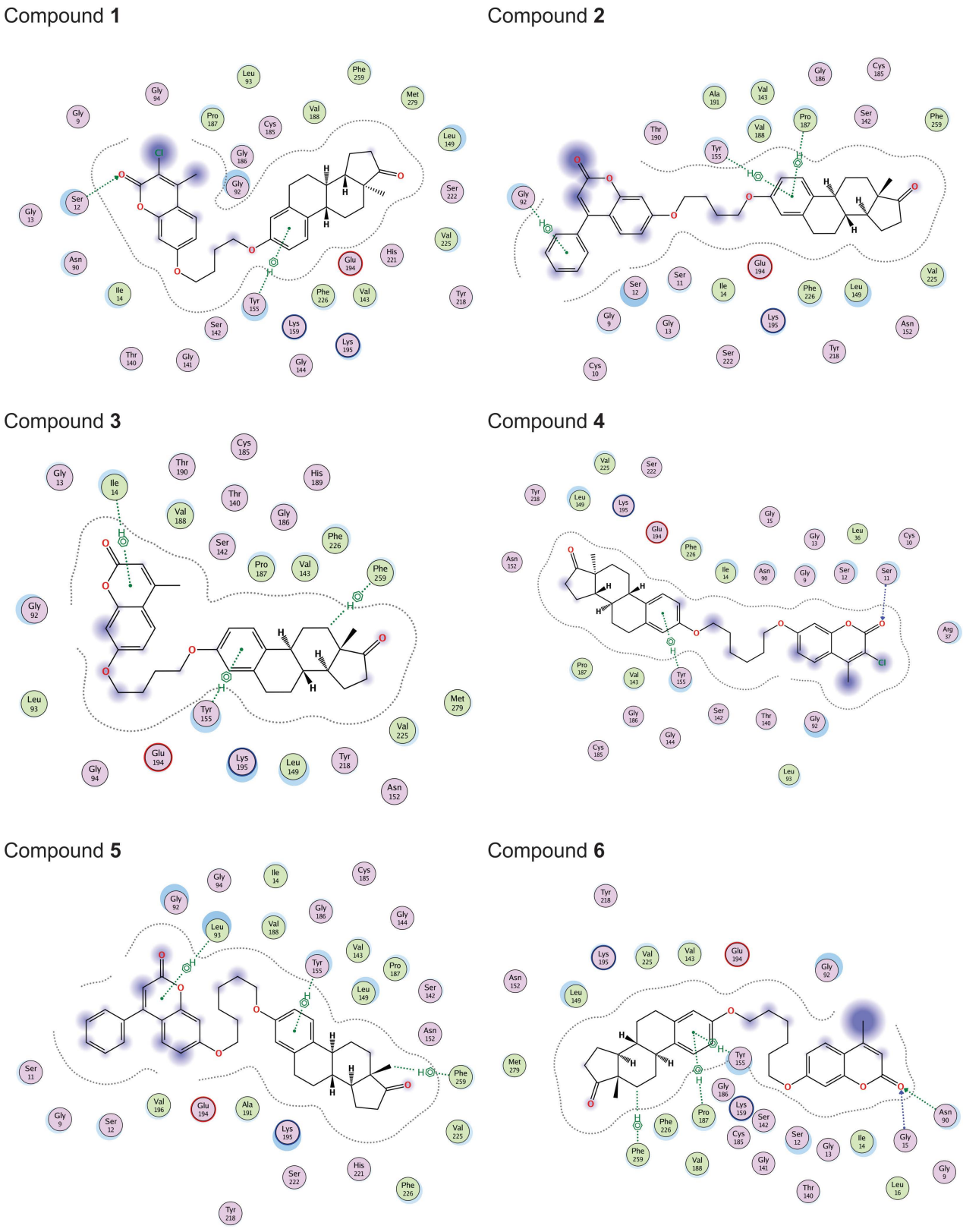

Figure 8. 2D interaction diagrams of Compounds **1** (ΔG = −8.9003 kcal/mol), **2** (ΔG = −8.9050 kcal/mol), **3** (ΔG = −8.6647 kcal/mol), **4** (ΔG = −8.8891 kcal/mol), **5** (ΔG = −9.5301 kcal/mol) and **6** (ΔG = −9.3532 kcal/mol) in the 17β-HSD1 crystal structure obtained with the MOE program.

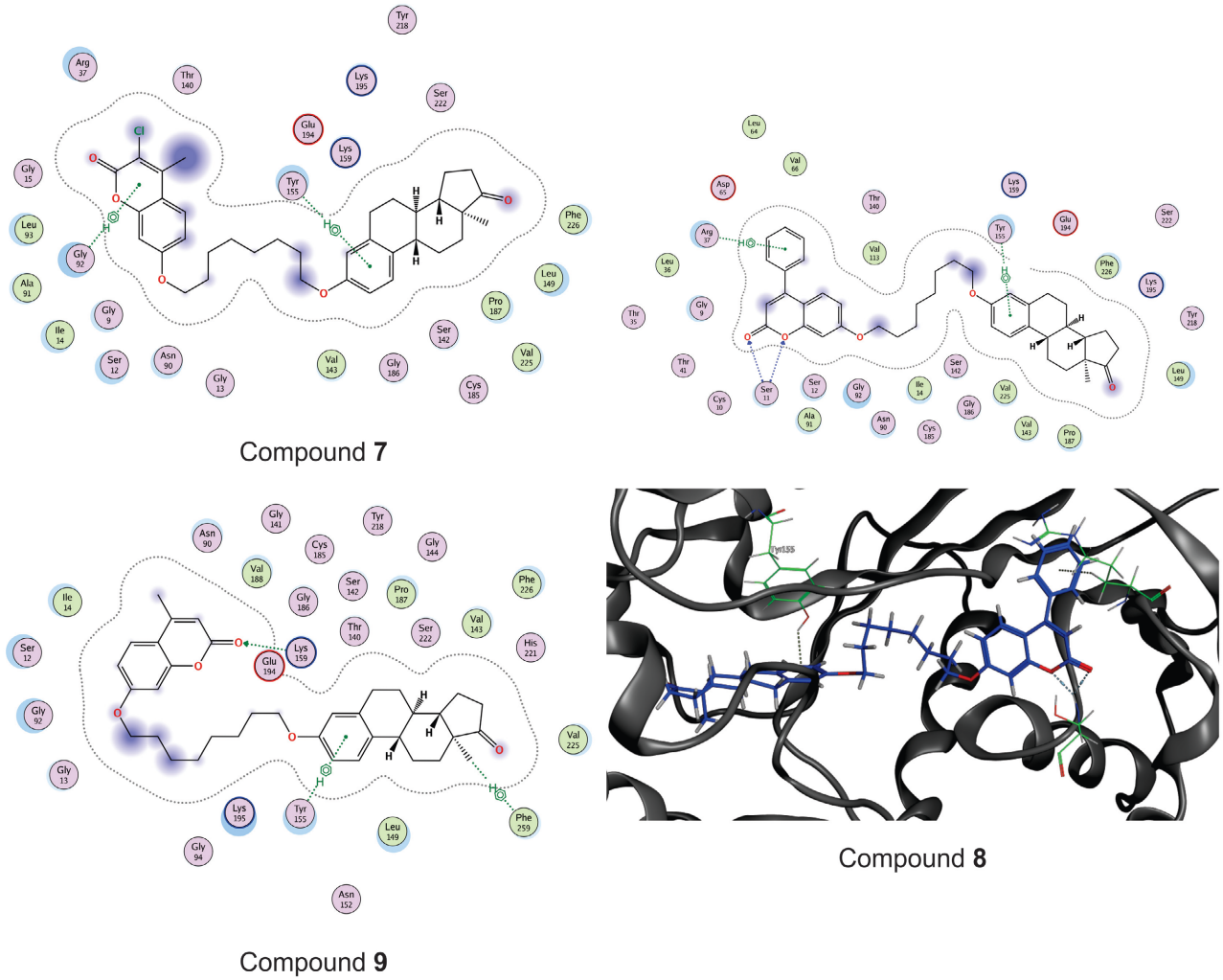

Figure 9. 2D interaction diagrams of Compounds **7** (ΔG = −10.2370 kcal/mol), **8** (ΔG = −11.3970 kcal/mol) and **9** (ΔG = −9.3219 kcal/mol) in the 17β-HSD1 crystal structure obtained with the MOE program.

Notably, the presence of the coumarin attached through variable chain lengths to the A ring of the steroidal moiety has cause a reverse orientation of the molecule in the active site, with the A ring facing the catalytic site (Tyr155), and D ring pointing to the recognition edge of the binding site (**Figure 8** and **Figure 9**). This inverse orientation causes inhibition of the enzyme, forming a dead-end complex that cannot be catalyzed. This type of inhibition, also reported in other enzymes, has been related with high concentrations of substrate [41] [42] [43] [44] [45].

In the conversion of E1 (estrone) to E2 catalyzed by 17β-HSD1, NADPH behave as a co-factor; at the cofactor binding site, NADPH is surrounded by the amino acids Gly9, Cys10, Ser11, Ser12, Ile14, Gly15, Arg37, Asn90, Gly92, and Lys195, forming important hydrogen-bonding interactions (**Figure 10**) [32]. It can be observed that for the products **1** - **9**, the aliphatic linkers together with the coumarin fragments are in the same NADPHs cofactor binding region. Furthermore, in Compounds **1**, **4**, **6**, **8** and **9**, the oxygens of the lactone portion act

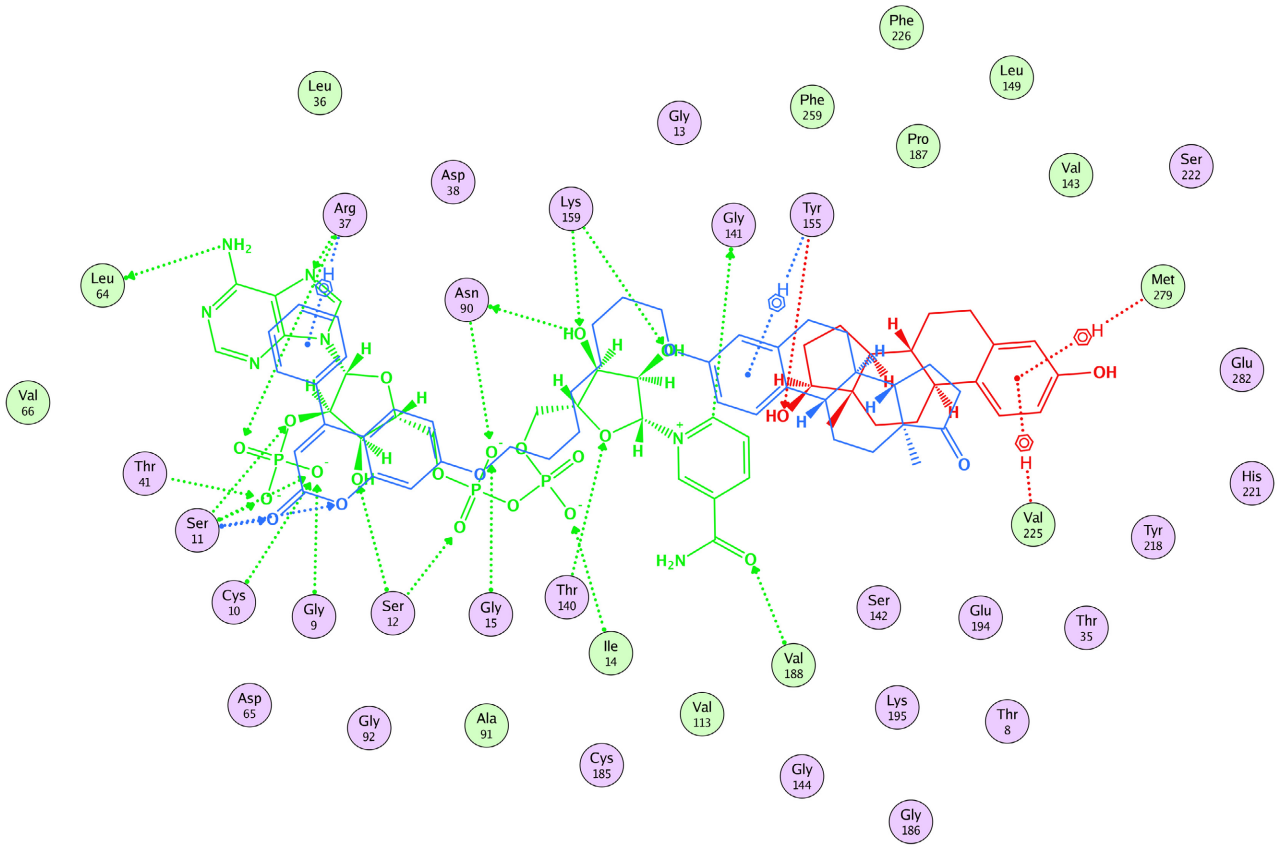

Figure 10. 2D overlay of the crystallographic ligand E2 (red), NADPH (green) and docked pose of Compound **2** (blue).

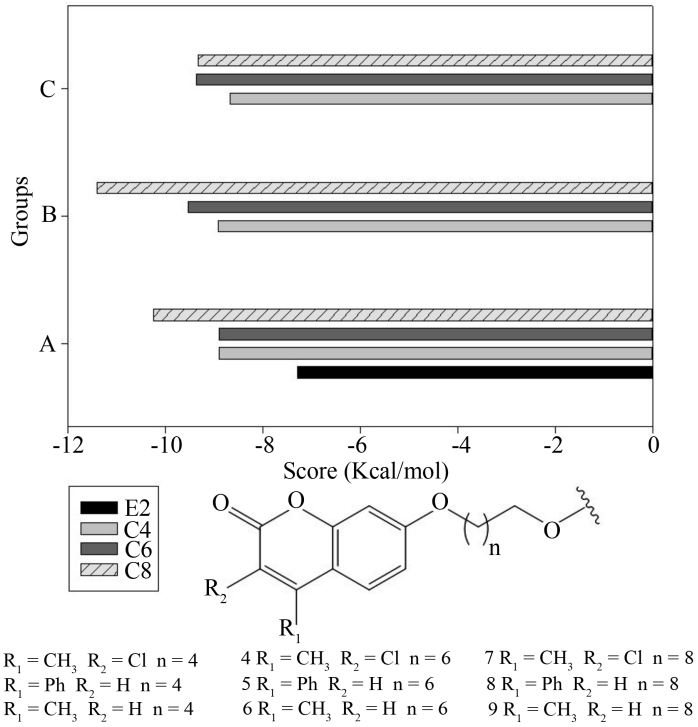

Figure 11. Effect of string length on score values. Group A: Compounds **1**, **4** and **7**; Group B: Compounds **2**, **5** and **8**; Group C: Compounds **3**, **6** and **9**.

as hydrogen bond acceptors of the residues Ser11, Ser12, Gly15, Asn90 and Lys195. Moreover, the electron-rich π cloud of the unsaturated lactone moiety of Compounds **3**, **5** and **7** interacted with hydrogen atoms (Δ+) of the amino acids Ile14, Leu93 and Gly92 in an arene-H interaction type. Additionally, Compounds **2** and **8** bearing a Ph group at the C4 position of coumarin, show an arene-H type interaction with Gly92 and Arg37 respectively (Figure 11).

Although all nine compounds obtained good scores compared to E2, as the methylene chain increased from 4 to 8 atoms, better scores were obtained, highlighting Compounds **7** and **8** with scores of −10.2370 kcal/mol and −11.3970 kcal/mol respectively (Figure 11).

4. Conclusions

The *in silico* analysis showed good score values in the proposed compounds, the steroidal portion presented important interactions with Met374 and Tyr155 in the catalytic site of aromatase and 17β-HSD1 respectively. Notably, Compounds **2**, **5** and **8** with an aromatic ring attached to C4 of the coumarin moiety, favored arene-H type interactions, essential for protein-ligand recognition. In addition, for the enzyme 17β-HSD1, it was observed how the length of the hydrocarbon chain influences in the number of binding points, being Compound **8** with a chain of eight methylenes, the one that presented the best score. This linker allows the steroidal portion to locate into the substrate binding site, while the coumarin portion interacts with the cofactor binding site with amino acids Arg37 and Ser11.

As a result of the analysis performed in this work, it can be concluded that the increment of the length of the linker up to 8 methylenes, optimizes the number of interactions at different sections of the enzyme. Moreover, it has been established an excellent starting point for the synthetic stage of these promising molecules as inhibitors of these two enzymes related to breast cancer (HR+). Molecular docking helped predict the binding mode of the designed Compounds **1 - 9** and their target proteins and predict the affinity and probable activity of the molecules. The synthesis and enzyme inhibition studies of these compounds will help to corroborate the most suitable structure against these molecular targets.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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