SYNTHETIC ANABOLIC STEROIDS BINDING
TO THE HUMAN ANDROGEN RECEPTOR*

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Within this study we assess the affinity binding of a few synthetic anabolic oral administrable steroids: oxymetholone, oxandrolone, methandrostenolone and stanozolol to the human androgen receptor (hARLBD) and a few nonspecific receptors. Molecular docking studies reveal that all these steroids are able to bind to the hARLBD and other nuclear and hormone receptors, despite the law sequence similarity between these receptors. The highest binding energy is registered for methandrostenolone binding to hARLBD, its molecular properties being the most similar to those of the natural ligand, testosterone. Stanozolol provides higher interaction energies for nonspecific receptors in comparison to its interaction with hARLBD. As the molecular properties of all investigated steroids are comparable, these results also illustrate that even small differences in the ligand properties have impact on the interaction strength. Also, computational characterization of structural properties of the hARLBD reveals that androgenic synthetic steroids binding cavity is highly hydrophobic suggesting their possible binding to hydrophobic cavities in various proteins in correlation with their observed side effects.

Key words: anabolic synthetic steroids, molecular docking, nonspecific interactions.

1. INTRODUCTION

Anabolic steroids are testosterone derivatives having two major functions: they are androgenic, controlling the male characteristics and they are anabolic regulating anabolic processes such as increasing the metabolism of ingested proteins and facilitating the synthesis of skeletal muscle, formation of blood cells, and the emotional and physical aspects of sexual function [1]. They are a class of drugs legally available only by prescription, but non-medical use of anabolic

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steroids is found especially in sports for performance improving, but also by young people obsessed with body image [2]. Anabolic steroids arrive in the nucleus, where they bind to the androgen receptor and are able to change the expression of a wide variety of genes, turning on numerous anabolic and androgenic functions [3]. So, there are many health risks from the use and abuse of anabolic steroids because they have a lot of side effects.

There are three structural files in the Protein Data Bank [4] concerning the structures of the human androgen receptor ligand – binding domain (hARLBD) in complex with two natural androgens (testosterone and dihydrotestosterone) and with an androgenic steroid used in sport doping (tetrahydrogestrinone) [5]. These files are the bases of molecular modeling studies and characterization of the steroids-receptor interactions.

The aim of this study is to assess the possible interactions between the considered synthetic anabolic steroids and the human androgen receptor ligand-binding domain and other human receptors using computational methods.

2. METHOD

The three structural files retrieved from the Protein Data Bank [4] concerning the structures of the human androgen receptor ligand-binding domain (hARLBD) in complex androgen steroids have the codes entry: 2AM9 for the structure of hARLBD in complex with testosterone (TST), 2AMA for the structure of hARLBD in complex with dihydrotestosterone (DTST) and 2AMB for the structure of hARLBD in complex with tetrahydrogestrinone (THT) [5]. This domain contains 266 amino acids, the region 654–919 of the human androgen receptor.

Fpocket tool [6] has been used to identify cavities or protrusions on the hARBLD surface and to characterize their local geometric and chemical properties in order to depict the important features necessary to bind anabolic steroids.

Four oral administrable synthetic anabolic steroids are considered in this study: oxymetholone (OXY), oxandrolone (OXA), methandrostenolone (MAS) and stanozolol (STA). The molecules of synthetic anabolic steroids have been extracted from ChemSpider chemical structure free database (http://www.chemspider.com/) using the Simplified Molecular-Input Line-Entry System (SMILES) linear formula [7]. They were used further as input by the Frog2 webserver [8] in order to obtain their three dimensional structures.

The UCSF Chimera software [9] is used to visualize, analyze and characterize the spatial structures of the protein and steroids and also to prepare them for molecular docking studies. The values of partition coefficients for the anabolic steroids are also extracted from ChemSpider database.

The computation of the interactions of anabolic steroids with hARLBD is performed using SwissDock web-based interface [10]. It yields the most favorable position and orientation of the ligand on the protein surface, which is called the binding mode, and the interaction energy expressed as:

$$E_{full} = E_{ligand} + E_{receptor} + E_{vdw} + E_{elec} + \Delta G_{elec,solv} + \sigma.SASA \qquad (1)$$

with E_{ligand} – the internal energy of the ligand, $E_{receptor}$ – the internal energy of the receptor, E_{vdw} – the van der Waals interaction energy, E_{elec} – the electrostatic interaction energy, $\Delta G_{elec,solv}$ – the electrostatic solvation free energy and $\sigma.SASA$ refers to the nonpolar contribution to the solvation energy [11]. He obtained binding modes are clustered and clusters are ranked by averaging the FullFitness scores of their elements. Visualization of the molecular docking results is performed using Chimera package [9].

Basic Local Alignment Search Tool (BLAST) algorithm has been used to identify sequence characteristics of the hARLBD that are possible present in other proteins [12] in order to assess nonspecific interactions and CLUSTALW tool [13] has been used to perform multiple sequence alignment.

STRING (Search Tool for the Retrieval of Interacting Genes) database has been also used to predict the interacting partners for the human androgen receptor [14].

3. RESULTS AND DISCUSSIONS

The three available spatial structures of hARLBD in complex with anabolic steroids have been superposed and we notice that there are only minuscule conformational changes from one complex to another.

Using Fpocket tool we have identified 16 cavities on the hARLBD surface and superposition of these cavities on the protein structure allowed to detect and characterize the testosterone binding cavity. Fig. 1 illustrates the superposition of structures of the complexes of the hARLBD with anabolic steroids and the contour of identified cavity is also schematically displayed. The proteins are shown as ribbons, the ligands as surfaces and the cavity contour as dashed lines, small spheres and sticks.

SwissDock on-line server has been used to assess the interactions between the considered anabolic steroids and hARLBD. All these steroids are able to bind in multiple places on the hARLBD surface and they are also proficient to bind to the same binding cavity as testosterone, this result being illustrated in Fig. 2.

The molecular properties of the natural ligand, TST, and of considered anabolic steroids OXY, OXA, MAS and STA are computed using CHIMERA tool and they are presented in Table 1 in addition to the partition coefficient (logP)

values and their computed interactions energies with hARLBD. The methandrostenolone possesses the highest binding affinity for hARLBD, its molecular properties being the most similar to those of the natural ligand, testosterone.

Fig. 1 – Superposition of structures of hARLBD (ribbons, 2AM9 – black, 2AMA – dark grey and 2AMB –light grey) in complex with anabolic steroids (surface, black, only TST being visible). The binding cavity delineation is marked using black dashed lines, small spheres and sticks.
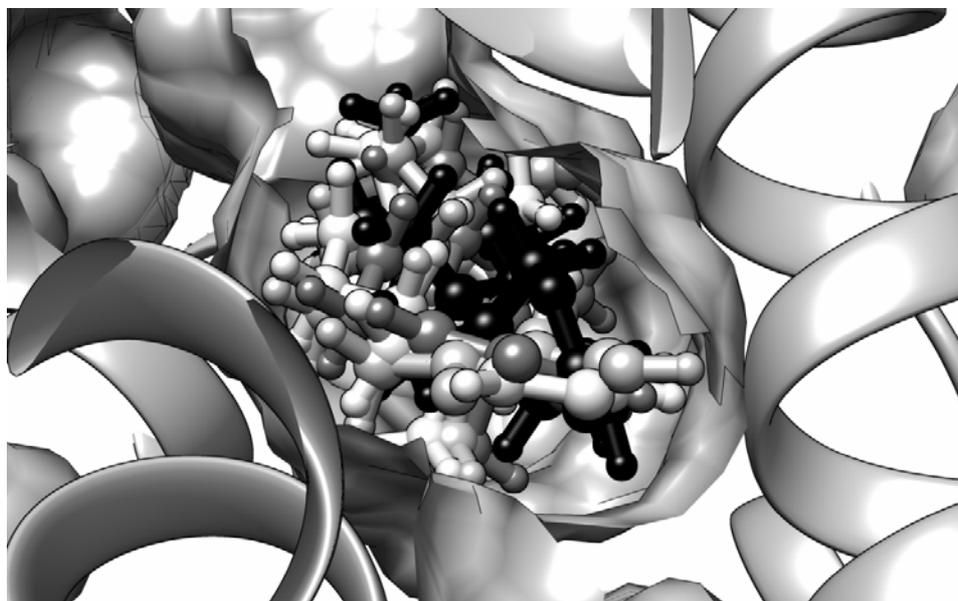

Fig. 2 – Binding of anabolic steroids to the active site of hARLBD: TST – black, OXY – dark grey, OXA – dim grey, MAS – grey and STA – light grey. The binding cavity is shown as grey surface. And the rest of protein as ribbon.

Table 1

Molecular properties of steroids and their interaction energies with the human androgenic receptor ligand-binding domain

Anabolic steroid	Molecular weight (Da)	Molecular surface ($Å^2$)	Molecular volume ($Å^3$)	logP	Interaction energy (kcal/mol)	ΔG (kcal/mol)
testosterone	288.42	244.4	263.2	3.27	−1400.63	−10.06
oxymetholone	332.48	276.1	306.2	4.22	−1350.53	−8.83
oxandrolone	306.42	251.9	278.7	3.33	−1359.39	−8.65
methandrostenol one	300.43	252.9	275.8	4.04	−1369.55	−9.34
stanozolol	328.49	274.9	304.0	5.53	−1356.99	−8.43

Characterization of the anabolic steroids binding cavity has been performed using Fpocket tool, its properties are presented in Table 2.

Table 2

Binding cavity characteristics

Volume ($Å^3$)	Polarity score	Hydrophobicity score	Local hydrophobic density score
1099.10	14	51.78	77.10

The values presented in Table 2 illustrate that binding cavity of testotesterone and synthetic anabolic steroids is big and highly hydrophobic, this observation being in good agreement with the hydrophobic character of steroids.

Starting from the hARLBD sequence, BLAST tool identifies putative conserved domains, as it is shown in Fig. 3. BLAST identified both specific and nonspecific hits belonging to the C-terminal ligand binding domain of nuclear receptors family: receptors of steroids, thyroid hormone, retinoids, cholesterol by-products, lipids and heme. As similar sequences usually rise in analogous structures, this result indicates that considered anabolic steroids might be able to nonspecific binding to all the nuclear receptors family.

Taking into account the results furnished by BLAST we have considered a few structural files of the binding domains of such possible nonspecific receptors: 1UOM for human estrogen receptor, 2BAW for human nuclear receptor, 2PIN for thyroid receptor and 3L0J for orphan nuclear receptor. We assessed the interactions of considered anabolic steroids with these targets and the results are presented in Table 4. All considered anabolic steroids are able to bind to these targets, some predicted interactions being stronger than their interaction with the human androgen receptor binding domain.
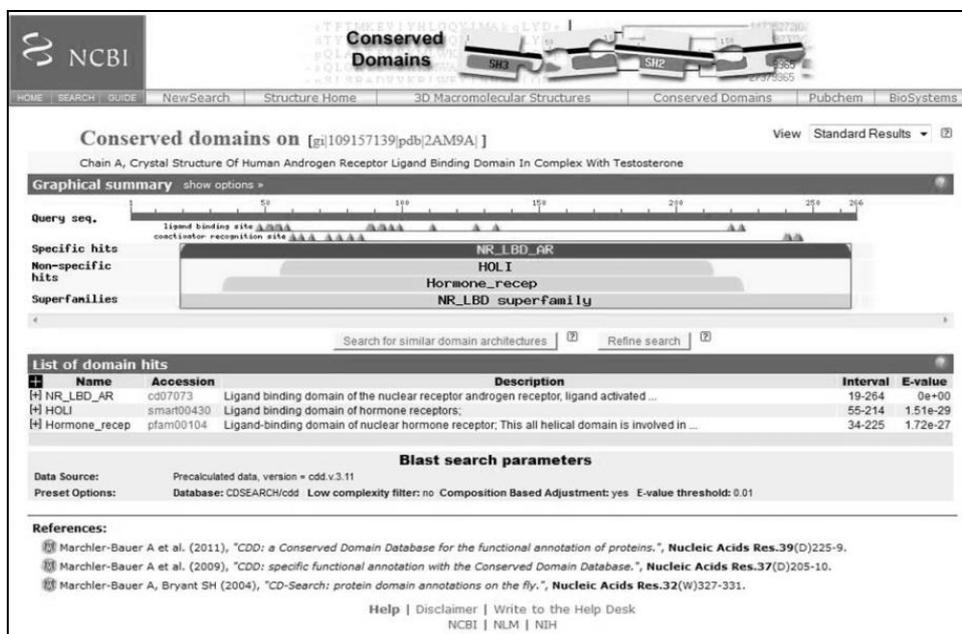
Fig. 3 – BLAST result for conserved domains containing the sequence of hARLBD.

Multiple sequence alignment of the human androgen receptor binding domain and considered nonspecific targets reflects a low sequence similarity, as presented in Table 3.

Table 3

Sequence similarity scores for considered proteins

Name	Name	Score
Human estrogen receptor	Human nuclear receptor	11.42
Human estrogen receptor	Human thyroid receptor	13.83
Human estrogen receptor	Human thyroid receptor	7.41
Human estrogen receptor	Human androgen receptor	20.08
Human nuclear receptor	Human thyroid receptor	21.34
Human nuclear receptor	Human thyroid receptor	20.99
Human nuclear receptor	Human androgen receptor	10.15
Human thyroid receptor	Human thyroid receptor	23.05
Human thyroid receptor	Human androgen receptor	11.46
Human orphan nuclear receptor	Human androgen receptor	10.7

Despite this law sequence similarity, the structural alignment (performed using *Structure matching* tool under Chimera package) reveals quite similar structures for the human androgen receptor and the nonspecific targets of anabolic steroids (Fig. 4).

Table 4

The interaction energies of considered steroids with nonspecific targets

| Anabolic Steroid/ Target	oxymetholone		oxandrolone		methandrostenolone		stanozolol	
	Fullfitness (kcal/mol)	ΔG (kcal/mol)	Fullfitness (kcal/mol)	ΔG (kcal/mol)	Fullfitness (kcal/mol)	ΔG (kcal/mol)	Fullfitness (kcal/mol)	ΔG (kcal/mol)
estrogen receptor	−1193.6	−6.87	−1202.1	−6.95	−1212.4	−7.87	−1236.2	−8.69
nuclear receptor	−1568.5	−7.79	−1575.6	−7.09	−1581.5	−7.39	−1596.5	−6.88
thyroid receptor	−1526.6	−7.75	−1539.8	−6.83	−1533.0	−6.91	−1551.2	−7.55
orphan nuclear receptor	−1357.7	−8.90	−1367.2	−8.71	−1368.6	−9.05	−1380.1	−8.46
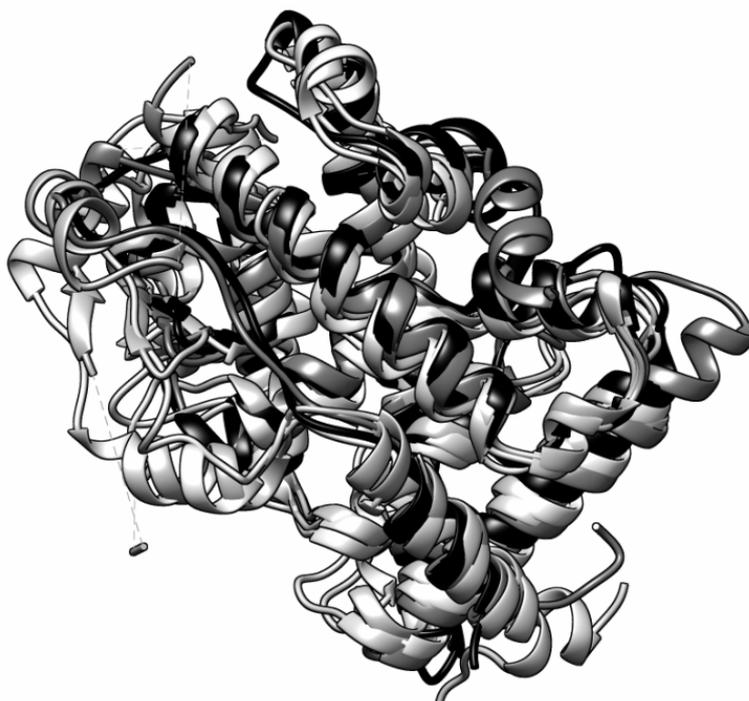
Fig. 4 – Structural alignment for the binding domains of the human androgen receptor (black), human estrogen receptor (dark grey), human nuclear receptor (dim grey), human thyroid receptor (grey) and human orphan nuclear receptor (light grey).

STRING tool predict with high confidence 62 interacting partners for the human androgen receptor and consequently, all these interactions might be influenced by the synthetic androgen steroids binding.

4. CONCLUSIONS

The results obtained within this study indicate that synthetic anabolic steroids are able to bind to the active site of hARLBD and also to another human nuclear and hormone receptors.

Among the synthetic anabolic steroids, the methandrostenolone possesses the highest binding affinity for hARLBD. Moreover, its molecular properties are the most similar to those of testosterone and this outcome illustrates that even small differences in the ligand properties significantly influences the interaction strength. For the nonspecific interactions, stanozolol possesses the highest binding energies for almost all the nonspecific targets, these energies being even higher than its interaction energy with hARLBD.

Another finding of our study is that testotesterone and synthetic anabolic steroids binding cavity is big and highly hydrophobic, suggesting that investigated steroids might bind to highly hydrophobic cavities of other proteins too.

Further computational and/or experimental studied are needed in order to assess the effects of anabolic steroids on the nonspecific targets in correlation with their side effects.

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