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Homology in the Binding Patterns of Human and Rat Androgen Receptors with various Ligands

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Abstract

Scientists routinely use *in-vivo* animal experiments to study the reproductive and endocrine effects of various chemicals in humans. Rats are being used as the most suitable animal model for such investigations. Use of animal models to envisage the mode of action of a particular chemical in humans is questionable unless we can explain the binding similarities. In this study, an *in-silico* docking was employed to visualise if androgens and their agonists bind with androgen receptors of humans and rats in a similar pattern using BIOVIA Discovery Studio 2018. Amino acid residues involved in bond formation, nature of bonding, LibDock score and bond distances were calculated to compare the binding affinities. It was found that ASN 705, GLN 711, ARG 752 and THR 877 were the major amino acid residues in hydrogen bonding of selected ligands with both human and rat androgen receptors. Thus, the present study answers numerous questions that may arise while selecting rats as laboratory animal models to validate the androgenic effects of chemicals in humans.

Keywords: Androgen Agonist, Androgen Receptors, Biovia Discovery Studio, *In-Silico* Docking, Laboratory Animal Models

1. Introduction

Androgen receptors (ARs) are the critical regulators of endocrine and reproductive functioning in males. They are members of the nuclear family of proteins possessing genomic and non-genomic actions[1]. ARs are soluble proteins with 919 amino acids[2]. Two isoforms of ARs (AR-A, 87kDa and AR-B, 110kDa) have been identified and characterized. The binding of endogenous androgens with AR induces conformational changes, including the hike in phosphorylation levels, homodimerization, nuclear translocation and interaction with DNA. The dimerized AR further binds with androgen response elements located at target genes and leads to cofactor recruitment, resulting in the regulation of androgen-dependent genes[1,2].

Dihydrotestosterone and testosterone are the major androgens produced in the human body[1]. These endogenous androgens possess numerous therapeutic effects too. These therapeutic activities are controlled either by upregulation or by downregulation of ARs. To meet the increased demands for androgens, derivatives were synthesized from endogenous androgens. These synthetic androgens can be androgen mimics (agonists) and androgen blockers (antagonists). Agonists and antagonists were, respectively, used for upregulation and downregulation of ARs. The use and abuse of synthetic androgens remain a highly debatable topic. Agonists aid in the treatment of male hypogonadism, aplastic anaemia, protein wasting diseases associated with cancer and so on[2]. Antagonists were developed for the treatment of prostate cancer, alopecia, hirsutism, etc. The abuse of androgen

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modulators by athletes and body builders has been widely reported[3,4]. There are also reports on the hidden side effects including hepatotoxicity, carcinogenicity, reproductive or developmental toxicity and cardiovascular toxicity[5].

Numerous reports on natural and synthetic compounds with androgenic/antiandrogenic potential have been published[6–11]. Anthropogenic chemicals with androgenic activity have also been reported to interfere with the physiological activities of aquatic organisms[12]. These reports created a growing concern about direct and indirect effects of compounds that bind with ARs. In order to screen the androgenic potential of chemicals, both *in vivo* and *in vitro* methods are used[13]. Hershberger's assay[14] and androgen receptor binding assay[15] are the most accepted protocols among them[16]. Ethical concerns, time consumption and financial input are the major limitations in these methodologies. This creates a huge urge for the development of a cost effective and faster methodology for investigating the agonistic and antagonistic activities of androgen receptors.

Research activities in development of newer methods for the investigation of androgenic effects of unknown chemicals are going on. Numerous *in vitro, in vivo* and *in silico* methodologies have been developed to screen androgenic potential of suspected compounds[17–21]. But none of these studies explained if the binding pattern of androgens with ARs of humans and experimental models are similar. Such comparative studies are relevant when we make conclusions about human effect of a compound based on animal studies. It was reported that amino acid sequences of human androgen receptors and rat androgen receptors shared an overall homology of about 85%[22]. But none of the reports till date has claimed that binding of a chemical with both human androgen receptors and laboratory animal androgen receptors are homologous. This could be achieved by manipulating the binding between a chemical and the corresponding biological receptor.

The present work is an investigation of homology in the binding patterns of selected androgenic compounds with ARs of rat and human. Four endogenous androgens and four androgen agonistic compounds were selected by literature survey. Discovery Studio 2018, a molecular docking software, was employed for molecular docking analysis. The LibDock score, amino acids involved in bond formation and the bond length were used for homology analysis. This is a pioneer attempt to study similarity in the binding patterns of human and rat androgen receptors. Additionally, this study also guarantees the validity of *in vivo* screening of androgenic activity of selected compounds.

2. Materials and Methods

- Molecular Docking: LibDock module of Discovery Studio 2018 was used to study the interaction between the protein and the ligand molecules. The binding sites were chosen based on PDB records for docking the ligands. The LibDock scores, nature of bonding and bond length of the docked ligands were estimated[23].
- **Ligands**: 3D structures of endogenous androgens and androgen agonists were downloaded from PubChem database in *.sdf* format. These ligands were prepared to generate 30 structures that included all possible conformers and tautomers. The list of ligands is presented in Table 1.
- **Receptors**: Androgen receptors that were bound with corresponding natural agonists were chosen for the purpose of docking. 3-D crystal structure of human androgen receptor (HAR) ligand binding domain in the complex with testosterone (PDBID: 2AM9)[24] and 3D crystal structure of rat androgen receptor (RAR) ligand binding domain complex with dihydrotestosterone (PDBID: 1I37)[25] were obtained from PDB (Protein Data Bank) (https://www.rcsb.org/) in *.pdb* format. The protein structure was cleaned (water molecules and other hetero-atoms removed), prepared and minimized before docking.

3. Results

All the selected ligands (endogenous androgens and androgen agonists) exhibited binding affinities with both Human Androgen Receptors (HAR) and Rat Androgen Receptors (RAR). LibDock scores, nature of bonding of amino acids and bond length are the significant parameters which specify the binding affinities. Tables 2 and 3 show the detailed information about the results of docking. It was interesting to see that all the four endogenous androgens (*androstendiol, dihydrotestosterone, epitestosterone, testosterone*) and four androgen agonists (*fluoxymesterone, methenolone, methyltrienolone, stanozolol*) selected for docking exhibited almost similar pattern of docking. 2-D docking images of all these ligands are clearly arranged in Figures 1 and 2.

Table 1. List of ligands selected for docking

Sl. No.	COMPOND NAME	CHEMICAL FORMULA	PUBCHEM ID	MOLECULAR WEIGHT	
1	ANDROSTENDIOL	$C_{19}H_{30}O_2$	10634	290.447	ENDOGENOUS ANDOGENS
2	DIHYDROTESTOSTERONE	$C_{19}H_{30}O_2$	10635	290.447	
3	EPITESTOSTERONE	$C_{19}H_{28}O_2$	10204	288.431	
4	TESTOSTERONE	$C_{19}H_{28}O_2$	6013	288.431	
5	FLUOXYMESTERONE	$C_{20}H_{29}FO_3$	6446	336.447	ANDROGEN AGONIST
6	METHENOLONE	$C_{20}H_{30}O_2$	3037705	302.458	
7	METHYLTRIENOLONE	$C_{19}H_{24}O_2$	261000	284.399	
8	STANOZOLOL	$C_{21}H_{32}N_2O$	25249	328.5	

LibDock scores, nature of bonding and bond length of endogenous androgens with HAR and RAR are clearly given in Table 2. RAR bounded to *Androstendiol* produced 6 hydrogen bonds (ASN 705, GLN 711, ARG 752, THR 877, LEU 704) and 7 hydrophobic interactions with a LibDock score of 111.389. HAR bounded to *Androstendiol* produced four hydrogen bonds (ASN 705, ARG 752, THR 877, LEU 704) and eight hydrophobic interactions, with a LibDock score of 102.317. RAR bound to *dihydrotestosterone* produced three hydrogen bonds (GLN 711, ARG 752, THR 877) and seven hydrophobic interactions, with a LibDock score of 109.724. HAR bound to *dihydrotestosterone* produced two hydrogen bonds (ARG 752, THR 877) and seven hydrophobic interactions, with a LibDock score of 102.506. RAR bound to *epitestosterone* produced four hydrogen bonds (ASN 705, GLN 711, ARG 752) and nine hydrophobic interactions, with a LibDock score of 107.884. HAR bounded to *epitestosterone* produced 3 hydrogen bonds (ASN 705, ARG 752) and 9 hydrophobic interactions, with a LibDock score of 99.9752. RAR bound to *testosterone* produced three hydrogen bonds (GLN 711, ARG 752, THR 877) and eight hydrophobic interactions, with a LibDock score of 109.874. HAR bound to *testosterone* produced two hydrogen bonds (ARG 752, THR 877) and seven hydrophobic interactions, with a LibDock score of 102.614. 2-D images of all these ligands are clearly arranged in Figure 1.

LibDock scores, nature of bonding and bond length of androgen agonists with HAR and RAR are clearly given in Table 3. RAR bound to *fluoxymesterone* produced four hydrogen bonds (GLN 711, ARG 752, THR 877, LEU 704) and seven hydrophobic interactions, with a LibDock score of 109.874. HAR bound to *fluoxymesterone* produced two hydrogen bonds (ARG 752, THR 877) and six hydrophobic interactions with a LibDock score of 113.916. RAR bound to *methenolone* produced three hydrogen bonds (GLN 711, ARG 752, THR 877) and nine hydrophobic interactions, with a LibDock score of 108.085. HAR bounded to *methenolone* produced two hydrogen bonds (ARG 752, THR 877) and nine hydrophobic interactions, with a LibDock score of 104.417. RAR bounded to *methyltrienolone* produced three hydrogen bonds (GLN 711, ARG 752, THR 877) and seven hydrophobic interactions, with a LibDock score of 107.511. HAR bound to *methyltrienolone* produced two hydrogen bonds (ARG 752, THR 877) and five hydrophobic interactions, with a LibDock score of 101.951. RAR bound to *stanozolol* produced two hydrogen bonds (MET 745, THR 877) and 14 hydrophobic interactions, with a LibDock score of 115.879. HAR bound to *stanozolol* produced three hydrogen bonds (GLN 711, THR 877, MET 745) and 14 hydrophobic interactions with a LibDock score of 114.907. 2-D images of all these ligands are clearly arranged in Figure 2.

Table 2. LibDock scores, nature of bonding and bond length of endogenous androgens with human and rat androgen receptors

COMPOUND	HUMAN ANDROGEN RECEPTOR			INTERACTING RESIDUES	RAT ANDROGEN RECEPTOR		
	LibDock SCORE	NATURE OF BONDING	BOND DISTANCE		BOND DISTANCE	NATURE OF BONDING	LibDock SCORE
ANDROSTENDIOL	102.317	Hydrogen Bond	2.06695	ARG752	2.05233	Hydrogen Bond	111.389
		Hydrogen Bond	1.99632	THR877	1.86427	Hydrogen Bond	
		-	-	GLN711	2.05233	Hydrogen Bond	
		-	-	GLN711	2.62213	Hydrogen Bond	
		Hydrogen Bond	2.67661	MET745	2.75892	Hydrogen Bond	
		Hydrogen Bond	4.327	LEU704	4.29526	Hydrogen Bond	
		Hydrophobic	5.28113	MET780	5.11726	Hydrophobic	
		Hydrophobic	4.67755	LEU873	4.5522	Hydrophobic	
		Hydrophobic	5.06221	LEU704	4.95481	Hydrophobic	
		Hydrophobic	4.4216	LEU707	4.2685	Hydrophobic	
		Hydrophobic	5.46528	MET742	5.31689	Hydrophobic	
		Hydrophobic	4.39684	MET745	4.10713	Hydrophobic	
		Hydrophobic	5.17701	PHE764	5.28065	Hydrophobic	
		Hydrophobic	5.20343	PHE764	-	-	
DIHYDROTESTERONE	102.506	-	-	GLN711	2.24901	Hydrogen Bond	109.724
		Hydrogen Bond	2.12124	ARG752	2.19863	Hydrogen Bond	
		Hydrogen Bond	1.94832	THR877	1.789	Hydrogen Bond	
		Hydrophobic	4.58143	LEU704	4.36315	Hydrophobic	
		Hydrophobic	5.15529	MET780	4.74837	Hydrophobic	
		Hydrophobic	4.18962	LEU873	4.2456	Hydrophobic	
		Hydrophobic	5.33127	MET742	5.18753	Hydrophobic	
		Hydrophobic	5.22046	MET742	5.25078	Hydrophobic	
		Hydrophobic	4.21534	MET745	4.1154	Hydrophobic	
		Hydrophobic	5.35755	PHE764	5.1076	Hydrophobic	
EPITESTOSTERONE	99.9752	Hydrogen Bond	2.12283	ARG752	2.31231	Hydrogen Bond	107.884
		Hydrogen Bond	2.36527	ASN705	2.03614	Hydrogen Bond	
		Hydrogen Bond	2.87947	ASN705	3.09305	Hydrogen Bond	
		-	-	GLN711	2.23371	Hydrogen Bond	
		-	-	THR877	2.63733	Hydrophobic	
		Hydrophobic	5.23795	LEU704	5.06762	Hydrophobic	
		-	-	MET780	5.45227	Hydrophobic	
		Hydrophobic	4.91387	LEU873	4.88424	Hydrophobic	
		Hydrophobic	5.44614	MET742	4.9553	Hydrophobic	
		Hydrophobic	5.06894	MET742	4.80238	Hydrophobic	
		Hydrophobic	4.66239	MET742	-	-	
		Hydrophobic	3.7541	MET745	3.62094	Hydrophobic	
		Hydrophobic	5.40249	TRP741	5.42611	Hydrophobic	
		Hydrophobic	5.08534	TRP741	5.24123	Hydrophobic	
		Hydrophobic	5.42335	VAL746	-	-	
TESTOSTERONE	102.614	Hydrogen Bond	2.12751	ARG752	2.31878	Hydrogen Bond	109.874
		Hydrogen Bond	1.94235	THR877	2.0607	Hydrogen Bond	
		-	-	GLN711	2.24135	Hydrogen Bond	
		Hydrophobic	4.63837	LEU704	4.95045	Hydrophobic	
		-	-	LEU704	4.91993	Hydrophobic	
		Hydrophobic	5.47901	MET780	5.29948	Hydrophobic	
		Hydrophobic	4.62599	LEU873	4.97212	Hydrophobic	
		Hydrophobic	5.35599	MET742	5.16729	Hydrophobic	
		-	-	MET742	4.78767	Hydrophobic	
		Hydrophobic	4.07817	MET745	3.69228	Hydrophobic	
		Hydrophobic	5.40423	TRP741	5.20441	Hydrophobic	
		Hydrophobic	5.4877	MET 742	-	-	

Table 3. LibDock scores, nature of bonding and bond length of synthetic androgens with human and rat androgen receptors

COMPOUND	HUMAN ANDROGEN RECEPTOR			INTERACTING RESIDUES	RAT ANDROGEN RECEPTOR		
	LibDock SCORE	NATURE OF BONDING	BOND DISTANCE		BOND DISTANCE	NATURE OF BONDING	LibDock SCORE
FLUOXYMESTERONE	113.916	Hydrogen Bond	2.12751	ARG752	2.31878	Hydrogen Bond	109.874
		Hydrogen Bond	1.94235	THR877	2.0607	Hydrogen Bond	
		-	-	GLN711	2.24135	Hydrogen Bond	
		Hydrophobic	4.63837	LEU704	4.95045	Hydrogen Bond	
		-	-	LEU704	4.91993	Hydrophobic	
		Hydrophobic	5.47901	MET780	5.29948	Hydrophobic	
		Hydrophobic	4.62599	LEU873	4.97212	Hydrophobic	
		Hydrophobic	5.35599	MET742	5.16729	Hydrophobic	
		-	-	MET742	4.78767	Hydrophobic	
		Hydrophobic	4.07817	MET745	3.69228	Hydrophobic	
		Hydrophobic	5.40423	TRP741	5.20441	Hydrophobic	
METHTNOLONE	104.417	Hydrogen Bond	2.11281	ARG752	2.06715	Hydrogen Bond	108.085
		Hydrogen Bond	1.96878	THR877	2.1691	Hydrogen Bond	
		-	-	GLN711	2.56771	Hydrogen Bond	
		Hydrophobic	4.52935	LEU704	4.35823	Hydrophobic	
		Hydrophobic	5.23482	MET780	5.2631	Hydrophobic	
		Hydrophobic	4.12751	LEU873	3.66753	Hydrophobic	
		Hydrophobic	5.43075	MET742	5.47522	Hydrophobic	
		-	-	MET742	5.23861	Hydrophobic	
		Hydrophobic	4.30786	MET745	4.15165	Hydrophobic	
		Hydrophobic	4.21551	LEU704	3.63332	Hydrophobic	
		Hydrophobic	3.55047	LEU707	3.63331	Hydrophobic	
		Hydrophobic	5.40248	PHE764	5.15369	Hydrophobic	
		Hydrophobic	5.45147	PHE764	-	-	
METHYLTRIENOLONE	101.951	-	-	GLN711	2.43529	Hydrogen Bond	107.511
		Hydrogen Bond	1.72739	ARG752	2.00845	Hydrogen Bond	
		Hydrogen Bond	2.01867	THR877	1.75388	Hydrogen Bond	
		Hydrophobic	4.63981	LEU704	4.33782	Hydrophobic	
		-	-	LEU704	5.00239	Hydrophobic	
		-	-	LEU704	4.96488	Hydrophobic	
		Hydrophobic	4.65018	LEU873	4.93379	Hydrophobic	
		Hydrophobic	4.95729	MET742	5.0529	Hydrophobic	
		Hydrophobic	5.09546	TRP741	-	-	
		Hydrophobic	5.12507	PHE764	4.87	Hydrophobic	
		-	-	MET780	5.20865	Hydrophobic	
STANOZOLOL	114.907	Hydrogen Bond	1.87489	THR877	1.71551	Hydrogen Bond	115.879
		Hydrogen Bond	2.34259	MET745	1.92497	Hydrogen Bond	
		Hydrogen Bond	2.5724	GLN711	-	-	
		-	-	PHE764	5.42068	Hydrophobic	
		Hydrophobic -	5.03315	LEU704	4.908	Hydrophobic	
		-	-	MET742	5.00458	Hydrophobic	
		Hydrophobic	4.53583	LEU873	4.70281	Hydrophobic	
		Hydrophobic	4.94746	MET780	5.181	Hydrophobic	
		-	-	LEU873	4.551	Hydrophobic	
		Hydrophobic	5.05634	MET742	5.49087	Hydrophobic	
		Hydrophobic	4.75317	MET742	4.49608	Hydrophobic	
		Hydrophobic	2.72614	MET745	4.22345	Hydrophobic	
		Hydrophobic	5.49922	TRP741	5.28586	Hydrophobic	
		-	-	PHE876	5.17925	Hydrophobic	
		Hydrophobic	4.75087	LEU707	5.47208	Hydrophobic	
		Hydrophobic	4.48392	MET745	4.88927	Hydrophobic	
		-	-	MET749	4.59073	Hydrophobic	
		Hydrophobic	3.94087	MET745	-	-	
		Hydrophobic	4.70628	MET745	-	-	
		Hydrophobic	5.26765	TRP741	-	-	
		Hydrophobic	5.49726	PHE764	-	-	
		Hydrophobic	4.84724	LEU707	-	-	

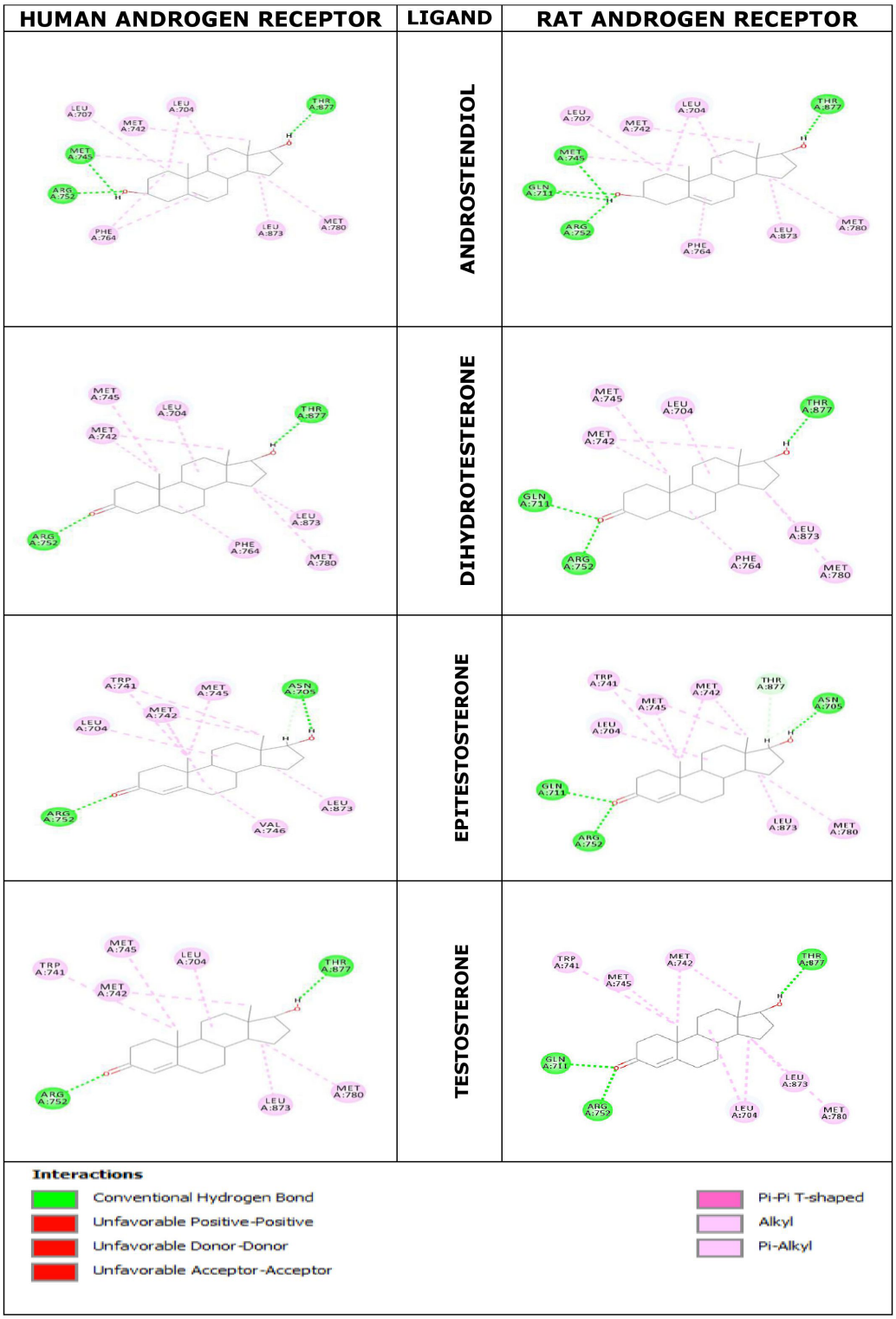

Figure 1. Two dimensional structures of binding of endogenous androgens with human androgen receptors and rat androgen receptors.

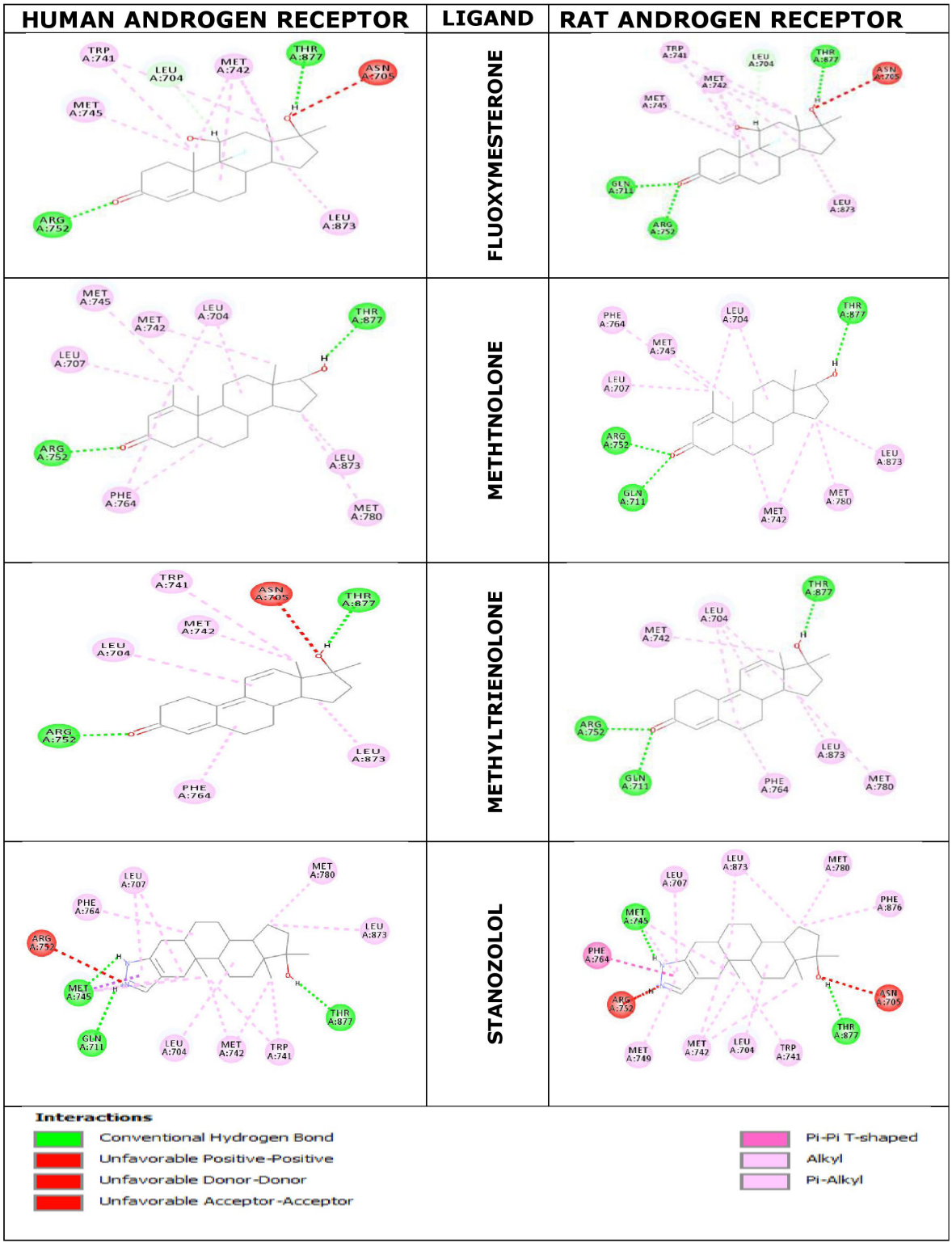

Figure 2. Two dimensional structures of binding of androgen agonists with human androgen receptors and rat androgen receptors.

4. Discussion

Several studies in animal models reported that the target genes of each AR are highly specific for the *in* vivo system. Experiments suggest that binding of ligands with AR is stabilized by the ligand-binding domain[26]. Different types of Androgen Response Elements (ARE) regulate AR activities[27]. Hormone-specific gene regulation is only possible due to the presence of these different mechanisms[28]. From our results, it is evident that both HAR and RAR exhibit similar binding patterns. The amino acid residues ASN 705, GLN 711, ARG 752 and THR 877 are mainly involved in hydrogen bonding in the selected ligands for both HAR and RAR. These amino acid residues are found to be the key regulators controlling the ligand-binding domains of AR[25,29,30,31]. Molecular docking helps to visualize that both humans and rats, these amino acid residues play crucial roles in binding ligands with AR. The crystalline structure of the HAR complexed with metribolone (R1881) revealed that ASN 705 and ARG 752 were the significant residues involved in forming hydrogen bonds with the ligand[30]. ASN 705, GLN 711 and ARG 752 in the LBD of HAR are involved in forming hydrogen bonds with dihydrotestosterone[25]. ASN 705 and THR 877 are involved in hydrogen bonding of 17-hydroxy group, and GLN 711 and ARG 752 are involved in hydrogen bonding of 3-keto group of testosterones with HAR[29]. Zhou *et al*[32] Made similar finding during screening of novel ligands for androgen receptors. Sakkiah *et al*.,[33] also reported that some of these amino acid residues were involved in binding of antagonists towards the antagonist binding pocket of the AR.

5. Conclusion

The present study revealed that both HAR and RAR exhibited similarity in binding patterns. Since both HAR and RAR shared homology in the binding patterns, we could predict that both human and rat have similar interactions towards the same compounds. This could also enable the prediction of similar physiological effects in both species towards the same compound. We also infer that this comparative study could be used as a reference for animal studies conducted for investigating toxicology and thus provides an additional validity for the *in vivo* results.

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