

## Research Article

## DESIGNING OF POTENTIAL NEW ESTROGEN ANTAGONISTS FOR TREATMENT OF ENDOMETRIOSIS: DESIGNING OF LIGANDS, MOLECULAR DOCKING, ACTIVITY, ADME & TOXICITY PREDICTION STUDY

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### ABSTRACT

The main objective of this study is to get more potent estrogen antagonist with greater safety profile. As estrogen antagonists and aromatase inhibitors both are generally used to treat endometriosis. But the large side effect profile of aromatase inhibitor are like Osteoporosis, increased coronary heart disease, and more over cost of treatment is more as compared with the treatment of estrogen antagonist. Due to many other factors these drugs have decreased compliance in patient use.

Among these estrogen antagonist like ethinylestradiol, in available preparations like Ovral-L and Novelon is used. Commonly used drugs in this patient subgroup are Clomifene and Zuclomifene, which have found greater acceptance. Clomifene and Zuclomifene are one of the widely used estrogen antagonists to control estrogen level during menstrual cycle. As endometriosis is a complex gynecological disorder that is characterized by the presence of endometrial tissues outside the uterine cavity, often in the peritoneal cavity. These endometrial cells respond to cyclic changes in hormonal milieu in a manner similar to the uterine uterine endometrial cell. Therefore we have designed some new estrogen antagonists by modification of Clomifene.

In this study we have designed some new estrogen antagonist and reported them as new potential estrogen antagonist by using various insilico tools and molecular docking analysis taking Clomifene as a prototype.

The modifications made in Clomifene have shown a better and safe profile as compared to Clomifene and it has been proved to have better binding affinity with estrogen receptor than Clomifene in docking study.

**Keywords:** Estrogen antagonist; Clomifene; Endometriosis; Molecular Docking; Activity Prediction; ADME & Toxicity Prediction.

### INTRODUCTION

Endometriosis is a disorder of estrogen production on a cellular level. It is a condition which generally occurs due to presence of endometrial tissue outside the uterine cavity and their response to cyclic changes in hormonal milieu in a manner similar to uterine endometrial cells during their reproductive age with a global impact of 6 to 10% of women annually [1,2,9]. It is the abnormal growth of cells (endometrial cells) similar to those that form the inside of the uterus, but in a location outside of the uterus. Endometrial cells are cells that are shed each month during menstruation. The cells of endometriosis attach themselves to tissue outside the uterus and are called endometriosis implants. These implants are most commonly found on the ovaries, the fallopian tubes, outer surfaces of the uterus or intestines, and on the surface lining of the pelvic cavity. They can also be found in the vagina, cervix, and bladder, although less commonly than other locations in the pelvis [3,4,5,9]. The main hormone responsible for this disease is estrogen and condition worsens with each menstrual cycle. Under the influence of menstrual female hormones each endometrial tissue grows and sheds blood at the time of menstrual cycle [9,10].

Estrogen is a female hormone synthesized in the theca interna of the female ovary and varies through the menstrual cycle. Estrogen is found to be at highest level at the end of follicular phase just before ovulation [16]. Estrogen promotes secondary sexual characters in female, like breast development. It also helps to maintain the thickening of endometrium and prepare uterus lining for implantation of fertilized ovary [17]. Highest level of estrogen is a major factor contributing to the growth and development of endometriosis. With the secretion of estrogen each endometrial tissue grows like cancer and cyst. These endometrial implants respond with great affinity to estrogen and they proliferate rapidly. This is due to a reason that endometrium implants produce a high level of estrogen and possesses high level of estrogen receptor. Endometriosis implants produce high level of estrogen as they contain an enzyme named aromatase which is found in endometriosis implants which converts the precursor of estrogen

into estrogen. These increased levels of estrogen contribute towards a growing factor of endometrium implants [18].

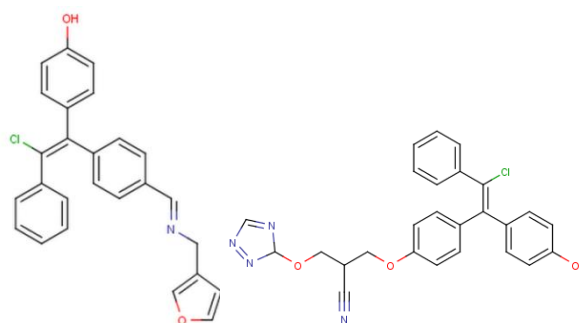
It has become a chronic disease nowadays and it can be controlled by estrogen antagonist as estrogen antagonist inhibits the action of estrogen and proliferation of endometrial cell is stopped [6, 13, 18]. The discovery of the first design of Estrogen antagonist drug has inspired a number of continuing efforts for the discovery of next generation drugs for endometriosis.

It was found that treatment of infertile women with Clomifene leads to congenital malformation; congenital heart disease creates a big problem in treatment [15]. In this study we have designed some new estrogen antagonists by modification of Clomifene which has been proved to possess better binding affinity with estrogen receptor than Clomifene in docking study.

### MATERIALS & METHODS

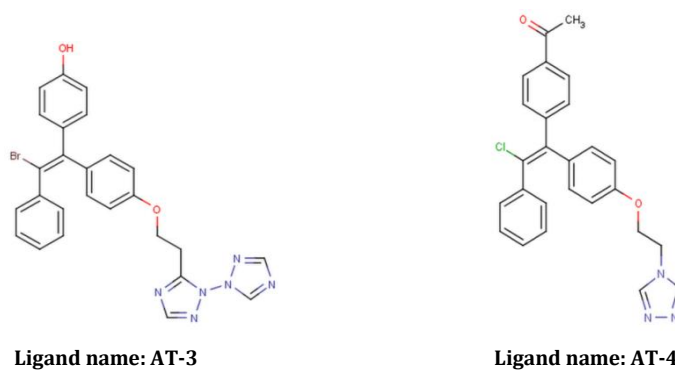
#### Designing of ligands:

Four ligands have been designed by structural modification of Clomifene which are then judged by docking study to predict their estrogen antagonist activity similar to that of Clomifene.

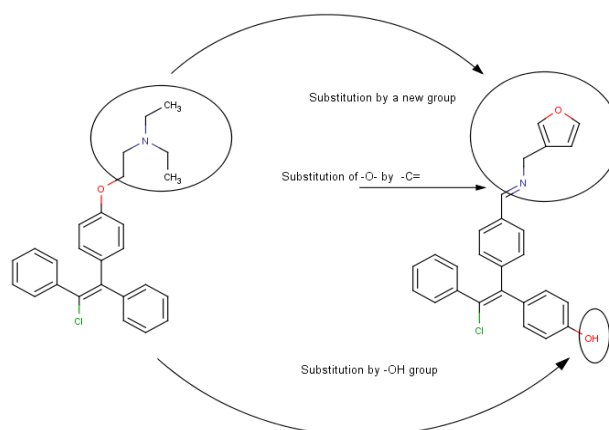


Ligand name: AT-1

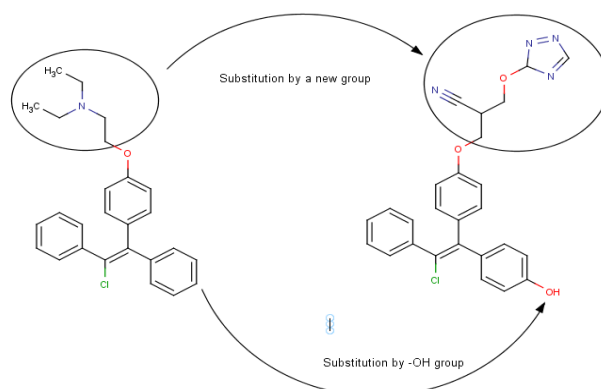
Ligand name: AT-2



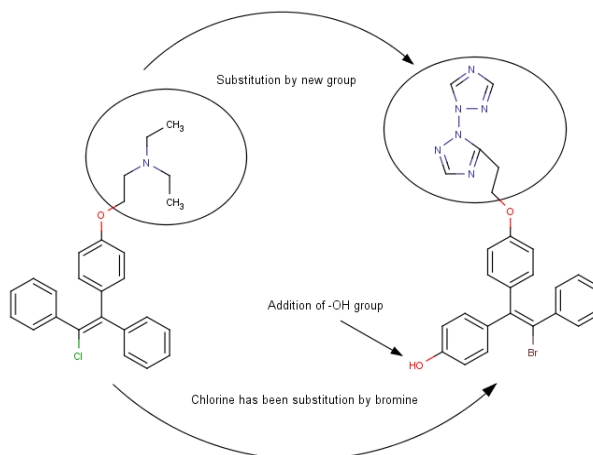
**Fig. 1: 2-D images of the designed ligands**



**Fig. 2: Comparison of Modification done on AT-1 with that of Clomifene**



**Fig. 3: Comparison of Modification done on AT-2 with that of Clomifene**



**Fig. 4: Comparison of Modification done on AT-3 with that of Clomifene**

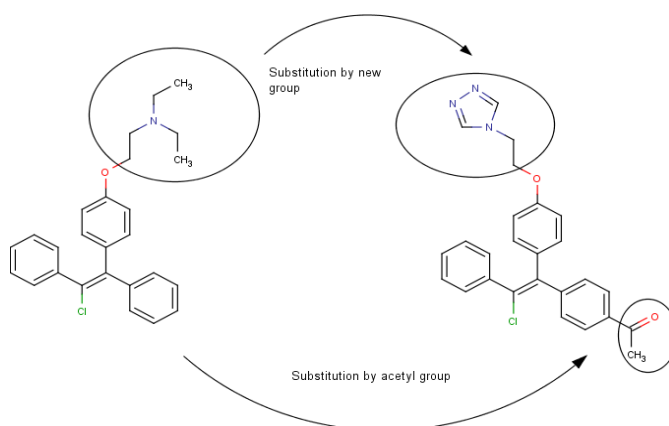


Fig. 5: Comparison of Modification done on AT-4 with that of Clomifene

### Molecular Docking Analysis

1-click docking [19] web based server has been used to dock the designed ligands with human estrogen receptor (PDB ID: 1R5K) [20]. 1-Click docking uses Vina filter and dock multiple ligands into a single target.

Default binding site centre specification of 1R5K in 1-Click Docking

Default binding site center X: 9.17

Default binding site center Y: 56.604

Default binding site center Z: 325.1457

### Activity Prediction

The activity of the designed ligands and Clomifene has been predicted using PASS Server [21] to confirm its Anti-fertility (female) and estrogen antagonist activity.

### ADME and Toxicity prediction

The AMDE/T properties of drugs, together with its pharmacological properties are conventionally viewed as a part of drug development. The best ligands after docking analysis were subjected to predict the pharmacokinetic properties using Pre-ADMET online server [22]. Structures with unfavorable absorption, distribution, metabolism and elimination have been identified as a major cause of failure of candidate molecule in drug development. Therefore early prediction of ADME properties has been done with the objective of increasing the success rate of the designed ligands in future development processes.

### Molecular Property Prediction

Molecular Property such as hydrogen bond donor (Donor HB) and hydrogen bond acceptor (Acceptor HB) has been predicted using MolSoft (<http://www.molsoft.com/mprop/>) [23] as molecular properties are essential for every stage of drug development from design to synthesis. Hence prediction of these parameters is important from the drug development point of view.

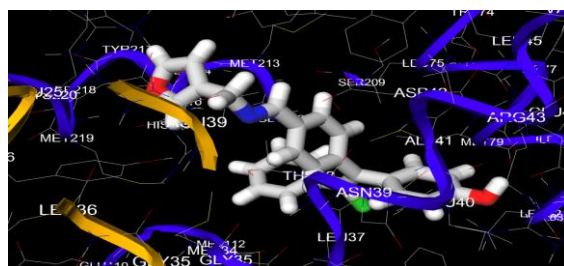
## RESULTS AND DISCUSSION

### Docking Analysis

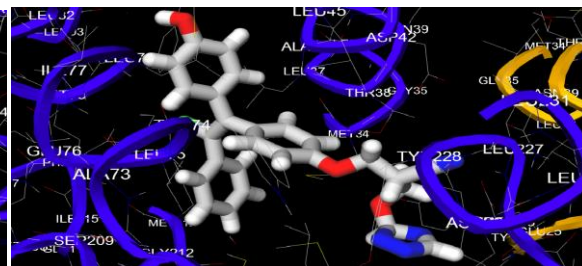
The molecular docking study of the designed ligands with human estrogen receptor (PDB ID: 1R5K) shows that all the designed ligands are showing better docking score than that of prototype Clomifene which predicts that the designed ligands have the better binding affinity with the estrogen receptor than Clomifene (Table 1). This prediction leads us to believe that the designed ligands will possibly be suitable for treatment of endometriosis.

Table 1: Docking score of the Designed Ligands and Clomifene with human estrogen receptor (PDB ID: 1R5K)

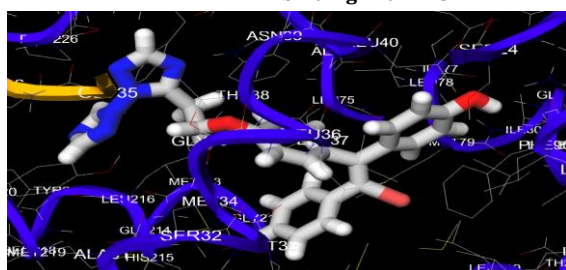
S. No.	Ligand name	Docking score
1	AT-1	-11.6
2	AT-2	-11.3
3	AT-3	-11.3
4	AT-4	-11.1
5	Clomifene	-10.2



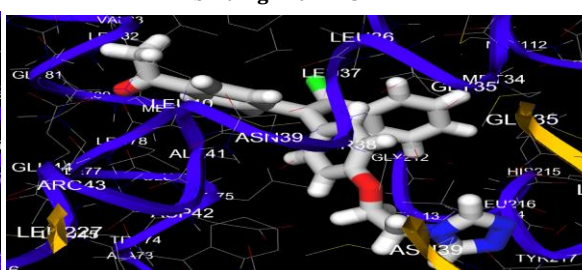
AT-1 binding with 1R5K



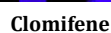
AT-2 binding with 1R5K



AT-3 binding with 1R5K



AT-4 binding with 1R5K



## Activity Prediction

been predicted using Pre-ADMET server and compared with the prototype molecule Clomifene which has been shown in Table 2 and Table 3

### Permissible ranges

Mol wt.: (130-725); Donor hb: (0.0-6.0); Accept hb: (2.0-20.0);  
%human intestinal absorption: 70-100 % well absorbed, 20-70%  
moderately absorbed, 0-20 poorly absorbed, %plasma protein  
binding: >90 strongly bound, <90% weakly bound

\*predicted using <http://www.molsoft.com/mprop/>

Sl. No.	Ligand name	Antifertility in female		Estrogen antagonist	
		Pa	Pi	Pa	Pi
1	AT-1	0,971	0,001	0,683	0,004
2	AT-2	0,974	0,001	0,546	0,004
3	AT-3	0,362	0,027	0,633	0,004
4	AT-4	0,976	0,001	0,660	0,004
5	Clomifene	0,988	0,001	0,851	0,003

\*Pa= Probability of Active, Pi=Probability of Inactive, Pa>Pi confirms significant activity

S. No.	Ligand Name	Donor HB*	Acceptor HB*	Mol. Wt (gm/mol)	%human intestine absorption	%Plasma protein binding
1	AT-1	1	3	413.12	96.988196	100.000000
2	AT-2	1	7	472.13	96.828827	95.861243
3	AT-3	1	6	528.09	97.240841	95.318652
4	AT-4	0	4	443.14	97.792734	92.909888
5	Clomifene	0	2	405.19	100.000000	92.964233

S. No.	Ligand name	Ames test	Carcinogenicity(Mouse)	Carcinogenicity(Rat)
1	AT-1	Mutagen	Negative	Positive
2	AT-2	Mutagen	Negative	Negative
3	AT-3	Mutagen	Negative	Positive
4	AT-4	Mutagen	Negative	Negative
5	Clomifene	Mutagen	Positive	Negative

negative result in carcinogenicity prediction in rat whereas the designed molecule AT-1 and AT-3 showed negative result in carcinogenicity prediction in mouse and positive result in carcinogenicity prediction in rat. Both of the above parameters are negative with the designed molecules AT-2 and AT-4. This predicts that AT-2 and AT-4 will have lower carcinogenic risk than that of prototype Clomifene.

Toxicity of all designed molecules have been predicted using PreADMET server and compared with the prototype drug Clomifene (Table 4). It is observed that in Ames test of all designed molecules as well as Clomifene shows mutagenic property. However Clomifene shows positive result in carcinogenicity prediction in mouse and

Clomifene is a potent estrogen antagonist which is being effectively used to treat Endometriosis. Our approach was to design the

molecules which is similar to that of Clomifene and which binds with more competence to the binding site of similar to Clomifene. Our study has given four molecules which demonstrate the better result in in-silico analysis with better binding efficiency (in terms of docking score) towards estrogen receptor than that of Clomifene. The docking result of AT-1, AT-2, AT-3 and AT-4 shows better docking score than that of Clomifene and AT-2 and AT-4 shows no trace of carcinogenic risk in toxicity prediction hence it has been predicted that all our designed ligands can possibly act as new leads for the treatment of endometriosis as they possess estrogen antagonistic activity.

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