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Research Article

DESIGNING OF POTENTIAL NEW AROMATASE INHIBITOR FOR ESTROGEN DEPENDENT DISEASES: A COMPUTATIONAL APPROACH

*Abhishek Thakur, Ajay kumar Timiri

Department of Pharmaceutical Sciences; Birla Institute of Technology, Mesra, Ranchi - 835215, India

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ABSTRACT

Aromatase inhibitor provides the best suitable approach at present for the treatment of many estrogen dependent diseases. The estrogen dependent diseases like breast cancer and endometriosis can be treated more effectively with new third generation aromatase inhibitors. As in case of breast cancer after mastectomy (removal of breast) regression of advance breast cancer is observed and in case of endometriosis even after total hysterectomy (removal of uterus and ovary) the reoccurrence of endometriosis is observed. So estrogen deprivation remains a main key as a therapeutic approach to cure estrogen dependent diseases. The third generation aromatase inhibitors are available in the preparations like Letrozole, Anastrazole, Vorazole and some more preparations are available. Among these preparations of aromatase inhibitor Letrozole is consider to be a better therapeutic agent. As it is found that patient using Letrozole as an aromatase inhibitor are having lower plasma estrogen level as compared to another third generation aromatase inhibitor and more over calcium reabsorption in bone is also seen with Letrozole. In this study we have designed some new aromatase inhibitor and reported them as potentially new aromatase inhibitor by comparing their pharmagological properties with Letrozole and some synthesized derivatives of Letrozole (Downloaded from Zinc data base with 90% structural similarity) (http://zinc.docking.org/search/structure) by using molecular docking analysis and various free internet based Insilco tools.

Key Words: Aromatase inhibitor, Letrozole, Estrogen dependent disease, Structure Activity Relationship (SAR), Zinc data base, Molecular docking, 3eqm pdb.

INTRODUCTION

Aromatase inhibitors are the inhibitor which provides a novel approach for the treatment of estrogen dependent diseases in post menopausal women by inhibiting the action of an aromatase enzyme. As aromatase is an enzyme which is responsible for the synthesis of estrogen by converting androgen into estrogen by a process called aromatization. Thus leading to an increasing in the rate of production of female hormone (estrogen) and this estrogen dependent diseases like breast cancer and endometriosis multiply rapidly in the presence estrogen thus making the condition worse[1,2,3,4,5,6,7]. As an aromatase inhibitor inhibits the action of aromatase enzyme hence on applying these aromatase inhibitors as a therapeutic agent leads to an increase in level of gonadotropin hormone due to negative feedback mechanism of estrogen hormone to hypothalamus and master gland (pituitary) [3,5,6,8,9,10]. Thus aromatase inhibitors are a class of drugs that block estrogen biosynthesis and controls estrogen level by negative estrogenic feedback at the pituitary.

Aromatase enzymes are expressed in high concentration in placenta and in the granular cells of ovarian follicles. This enzyme is also present in several non glandular tissues like endometrial tissues, breast cancer tissue, normal breast tissue, subcutaneous fat, muscle, liver and brain [4,6]. As we know that estrogen is also secreted from endometrial tissues, breast tissue and breast cancer tissues due to the presence of this aromatase enzyme, hence making the conditions favorable for endometrial and breast cancer tissues to multiply. Due to wide spread of these aromatase cells it is very difficult to control it by oophorectomy,

***Corresponding Author Address:** Abhishek Thakur, Department of Pharmaceutical Sciences; Birla Institute of Technology, Mesra, Ranchi -835215, India; E-Mail: thakurabhishek242@gmail.com*

hysterectomy or mastectomy. So estrogen deprivation remains the therapeutic approach to cure these estrogen dependent diseases [5,6,11,12,13]. As breast cancer contribute 22.9% of all cancers in women worldwide. It also affects one out of eight women during their whole life [14]. Breast cancer originate from breast tissue and we know that breast cancer tissue and normal breast tissue both are supplied with aromatase cell which secrete aromatase enzyme and catalyzes the formation of estrogen thus leading to rapid multiplication of breast cancer tissue. Some factors like abortion also increase the chance of breast cancer. As due to pregnancy the estrogen level is increased for the growth of breast to make it suitable for lactation. But after as Type-4 lobules of breast mature only after trimester, that are cancer resistant. Thus, gives resistance to women from breast cancer. Hence if before first trimester termination of pregnancy occurred due to some reasons like induced abortion, miscarriage or still birth then this process is disturbed and influence the chances of development of breast cancer. Many other factors like late age of menopause and early menarche also contribute towards the development of breast cancer [11,17,18]. As mastectomy (removal of breast) is not giving complete cure because breast cancer develop from the inner lining of milk lobules (lobular carcinoma) and milk ducts (ductal carcinoma) and these ducts and lobules are not only limited till breast tissue, but they also extends from collar bone to the lower rib and from the middle of the chest, around the side and under the arms. Hence making removal of every and every cancerous milk duct and lobules near to impossible by a surgical process known as mastectomy [13,17]. All this point is giving clear evidence that breast cancer is completely an estrogen dependent disease which can't be completely cured my various surgerical processes, as reoccurrence can happen. So estrogen deprivation remains a main key as a therapeutic approach for the treatment of estrogen dependent disease (Breast Cancer) which can be achieved by aromatase inhibitor and moreover it should be considered as a main path for the treatment of such disease [1,5,7].

It is estimated that endometriosis generally affects 6 to 10% of women in their reproductive age and 2-5% of women in postmenopausal age. Endometriosis is a disorder of estrogen production on a cellular level. It is a complex gynecological disorder that is characterized by the presence of endometrial tissues outside the uterine cavity, often in the peritoneal cavity and generally worsens with each menstrual cycle [18,19,20]. 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 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. This endometriosis implants are "Self Perpetuating" as they can regenerate and spread outside the uterus and ovary by self stimulation. This is a reason that reoccurrence of endometriosis occurs even after total hysterectomy (removal of uterus and ovary) as they remove endometriosis implants from uterus and ovary only. It has become a chronic disease nowadays [16]. So endometriosis is also an estrogen dependent disease which is not fully curable by surgical methods. Hence deprivation remains a main key as a therapeutic approach for the treatment and it can be achieved by aromatase inhibitor with better fruitful result [21,24].

The third generation aromatase inhibitor which is having fewer side effects on treating postmenopausal women and can be exploited in future for treating premenopausal women [8,22,23]. They have been designed earlier using the concept of rational drug design and the use of computer aided drug design tools. As the treatment of estrogen dependent disease with aromatase inhibitor has shown less side effect and increase in rate of ovulation as compared with the estrogen antagonist [10]. So to make this treatment more novel and effective with aromatase inhibitors we have designed some new ligands by taking Letrozole and some synthesized derivatives of Letrozole (Downloaded from Zinc data base with 90% structural similarity) (http://zinc.docking.org/search/structure) as a prototype. Ligands effectiveness is judged by computer aided drug design approach. Current computer aided molecular docking approach has been used in this study to judge binding efficiency of new ligands with that of aromatase inhibitor receptor.

Designing of ligands: Five ligands have been designed by structural modification of Letrozole which are then judged by docking study to predict their aromatase inhibitor activity as compared with Letrozole and some synthesized derivatives of Letrozole (Downloaded from Zinc data base with 90% structural similarity) (http://zinc.docking.org/search/structure).

Protein preparation: The 3D structure of our protein is available in Protein Data Bank. Crystal structure of human placental aromatase cytochrome P450 in complex with androstenedione (PDB ID 3EQM) is selected for performing the docking studies [25]. The selected protein 3D structure is having 1 chain; hence chain A was used for docking study. The 3eqm Pdb contains the structure of Cytochrome P450 19A1. It also contains heterocyclic compounds like ASD (4-Androstene-3-17-dione), Hem (Protoporphyrin IX containing FE) and PO_4 (Phosphate ion).

Molecular Docking Analysis: Autodock is used for the Molecular docking studies of the ligands with the receptor protein. Autodock uses binding free energy evaluation to find the best binding mode. Autodock energy values were calculated by the characterization of intermolecular energy (consist of van der Walls energy, hydrogen bonding energy, desolvation energy, and electrostatic energy), internal energy of ligand, and torsional free energy. The designed ligands were subjected to molecular docking studies and the docked complex is visualized using Python molecule viewer[26] as shown in (fig.10). Binding energy, ligand efficiency, inhibition constant, hydrogen bond interactions and their bond lengths are calculated as shown in (table 1).

Activity Prediction: The activity of the designed ligands and Letrozole has been predicted using PASS Server [27] to confirm its Anti-fertility (female), Aromatase inhibitor, Cytochrome P450 inhibitor and CYP19 inhibitor.

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 admetexp and Lazar server [28,29]. 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 server [30] 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: Docking score of Letrozole was found to be better as compared to synthesized derivatives of Letrozole (Downloaded from Zinc data base with 90% structural similarity). So further study was carried out by taking leterozole as a prototype and some ligands were designed and there docking and pharmacological studies were carried out.

Activity Prediction: The activity prediction of all the designed ligands has been done using PASS server and compared with Letrozole. It was found that designed ligands as well Letrozole when uploaded in the server as MOL format shows the significant properties like "Antifertility (female), Aromatase inhibitor, Anti-neoplastic and Anti-neoplastic (Breast cancer)" which predicts that the designed ligands will probably be effective to treat estrogen dependent diseases like breast cancer and endometriosis as that of Letrozole, as shown in table (3). But NA-3, NA-4 and NA-5 has also shown the activity of Calcium regulator.

ADME and Toxicity prediction: As per the ADME and toxicity prediction data, it has been predicted that all the designed ligands possibly be well absorbed through blood and intestine and non toxic. They will have similar type of plasma protein binding profile as that of Letrozole. This shows that the designed ligands are as per with the prototype Letrozole in terms of ADME and toxicity parameters and can be used in future as an aromatase inhibitors to treat estrogen dependent diseases, as shown in table (4).

As per the carcinogenicity prediction of designed ligand with our prototype Letrozole, designed ligands were found to be non carcinogenic. Moreover designed ligands (NA-1, NA-2 and NA-5) were found to be mutagenic, as shown in table (5).

Molecular Property Prediction: The drug-likeness and the molecular properties of the designed ligands are analyzed using Molsoft server and the designed ligands NA-1 and NA-2 presented better drug-likeness values than our prototype Letrozole**,** along with more number of hydrogen bond acceptor and donor. Stereo centers were also found in all designed ligands which is lacking in our prototype, as discussed in table (6).

CONCLUSION

Letrozole is a potent aromatase inhibitor which is being effectively used to treat estrogen dependent diseases. Our approach was to design the molecules

which is similar to that of Letrozole and which binds with more competence to the binding site of Letrozole. Our study has given five molecules which demonstrate the better result in in-silico analysis. During docking better binding energy was observed with aromatase inhibitors than that of Letrozole and some synthesized derivatives of Letrozole (downloaded from Zinc data base with 90% structure similarity). Moreover NA-5 has shown good hydrogen bonding which was not observed in our prototype Letrozole. All the designed ligands have shown no trace of carcinogenic. Moreover NA-1 NA-2 and NA-5 have shown mutagenic property. Hence it has been predicted that all our designed ligands can possibly act as new leads for the treatment of estrogen dependent diseases like endometriosis and breast cancer.

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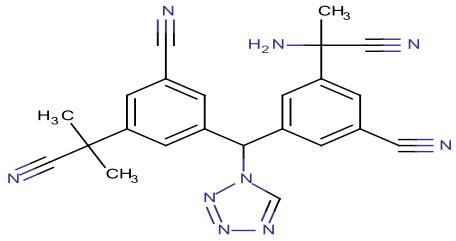
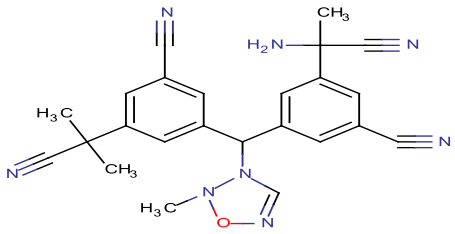
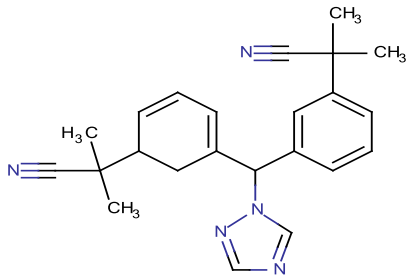


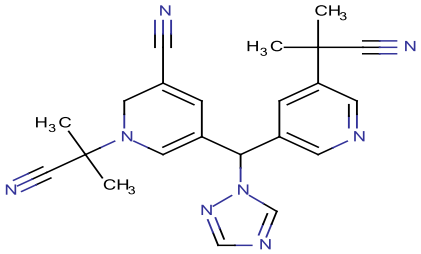
Ligand name NA-1



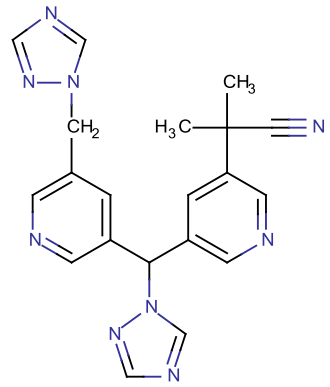
Ligand name NA-2



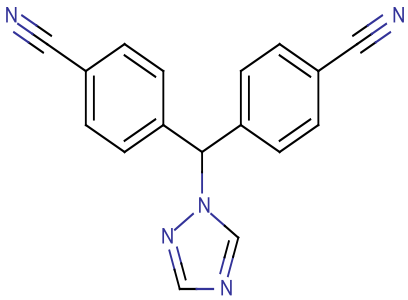
Ligand name NA-3



Ligand name NA-4



Ligand name NA-5



Letrozole

Fig.1 2-D images of the designed ligands and Letrozole.

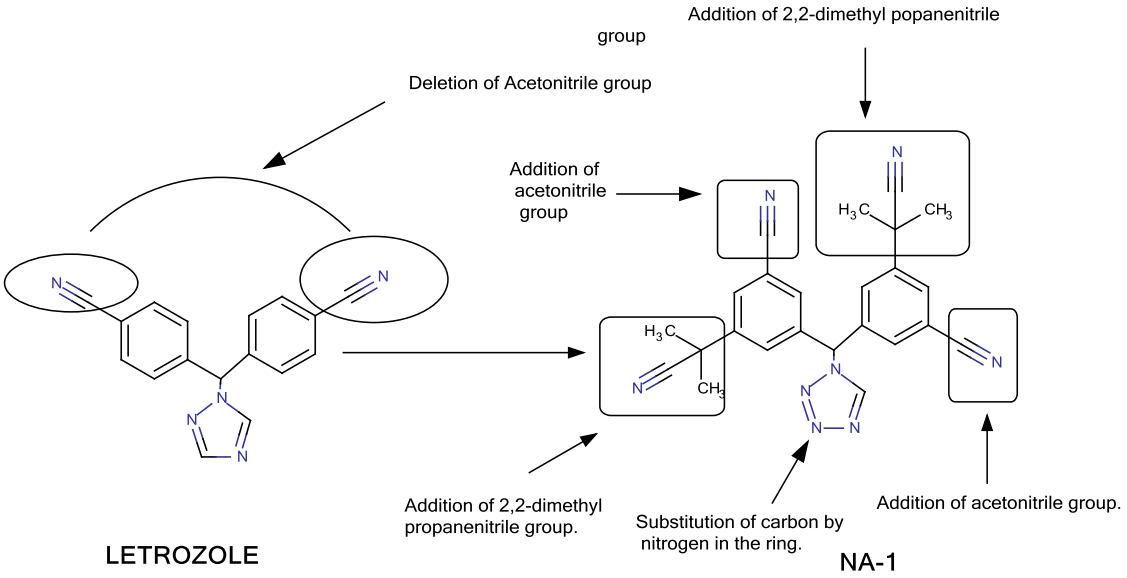


Fig.2 Comparison of Modification done on NA-1 with that of Letrozole

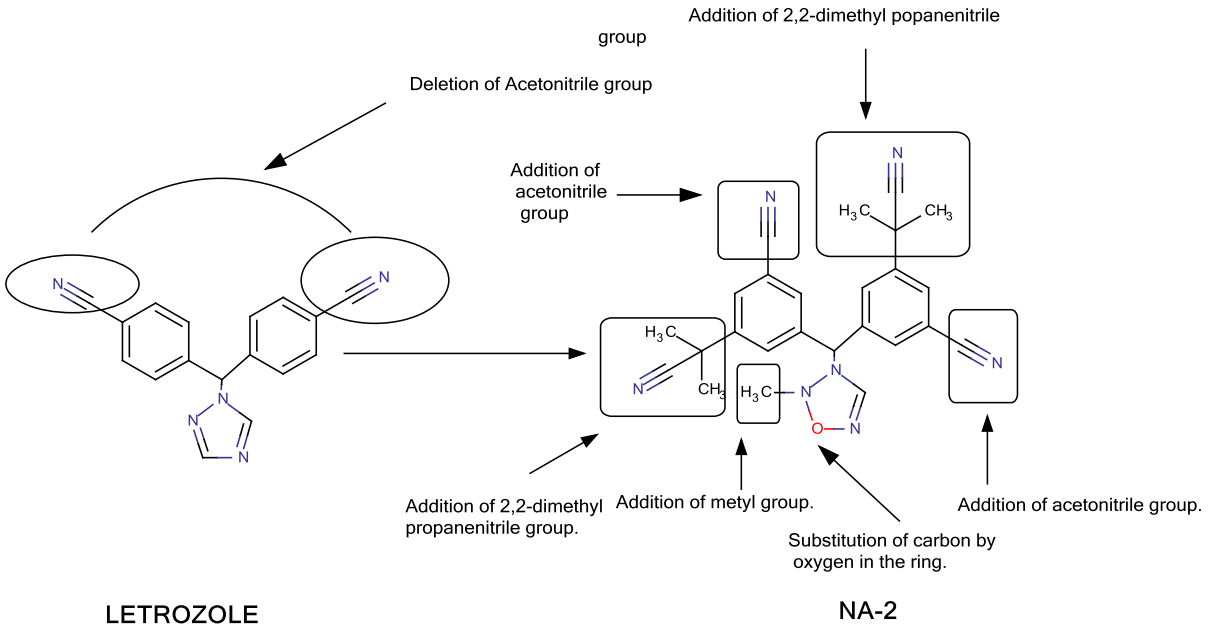


Fig.3 Comparison of Modification done on NA-2 with that of Letrozole

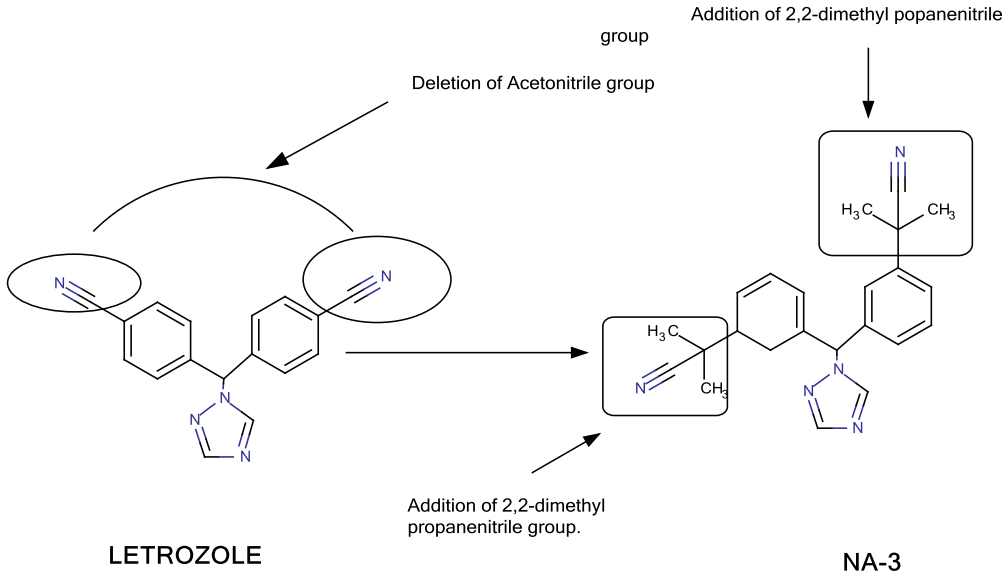


Fig.4 Comparison of Modification done on NA-3 with that of Letrozole

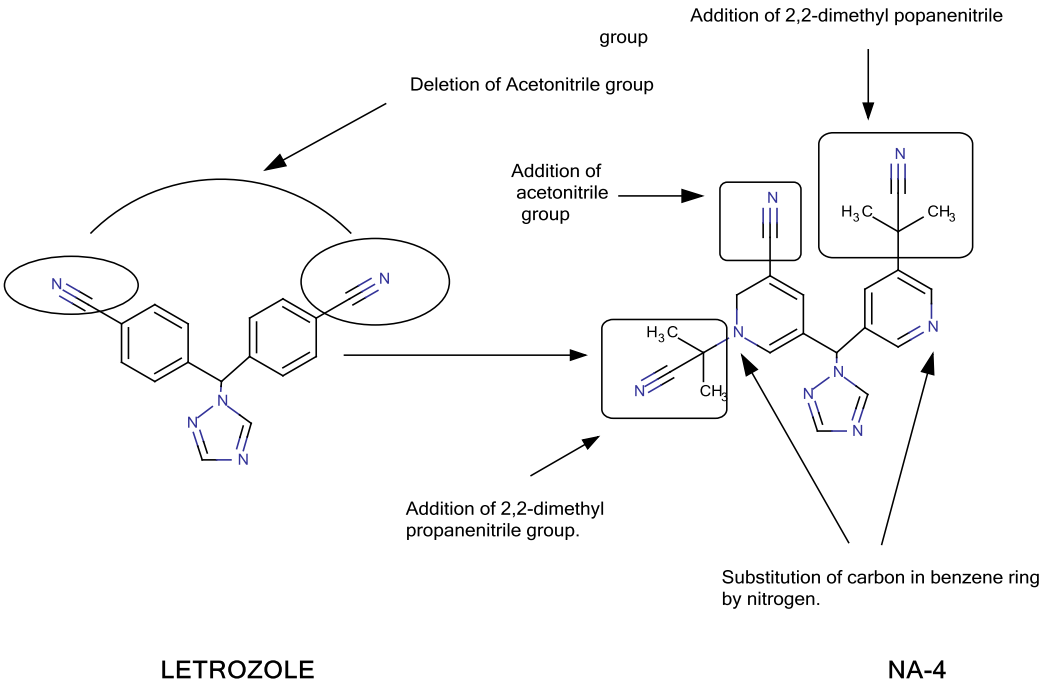


Fig.5 Comparison of Modification done on NA-4 with that of Letrozole

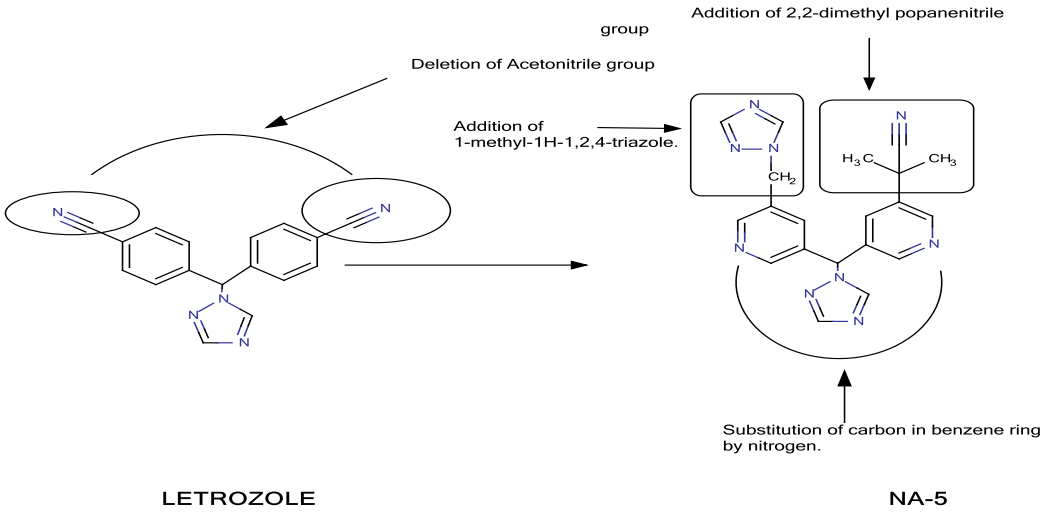
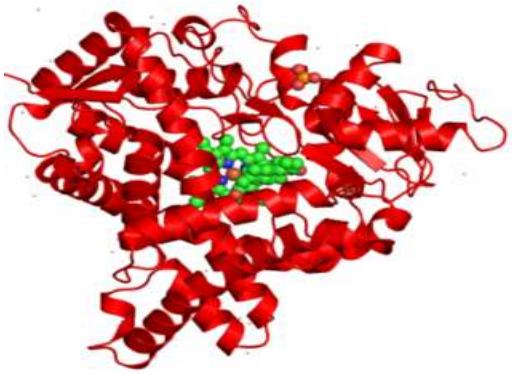
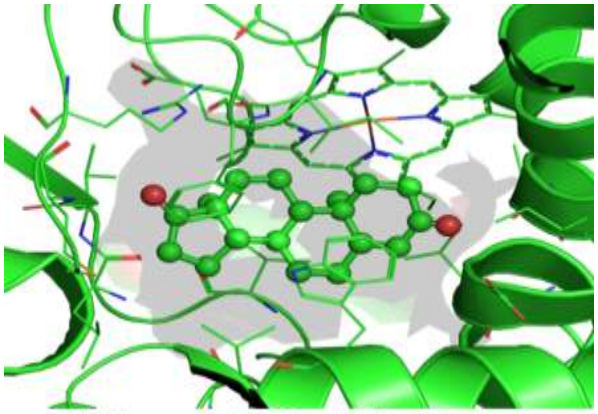


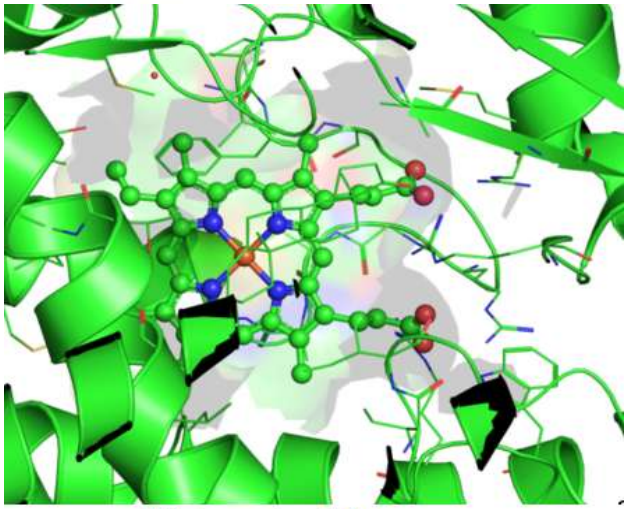
Fig.6 Comparison of Modification done on NA-5 with that of Letrozole



7(b). 3eqm pdb with heterocyclic compounds



7(c). 3eqm pdb with ASD at binding site.



7(d). 3eqm pdb with Hem.

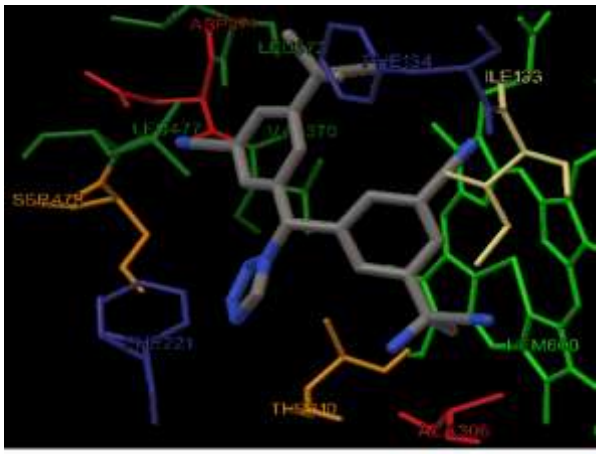
Fig.7 Structure of protein 3eqm with heterocyclic compounds at its binding site (Downloaded from server: http://www.ebi.ac.uk/pdbe-srv/view/entry/3eqm/summary)

Table.1. Docking result of some synthesized derivatives of Letrozole (Downloaded from Zinc data base with 90% structural similarity) taking Letrozole as a prototype with aromatase inhibitor PDB ID: (3EQM)

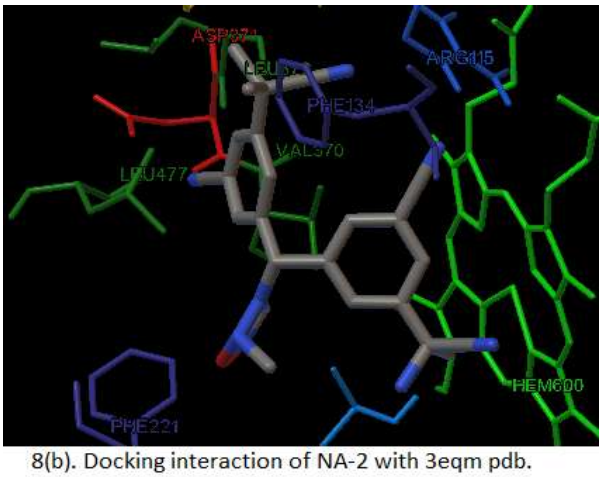
Sl.no:	Name of the Ligand	Binding energy (K.Cal/Mol)	Ligand efficiency	Inhibition constant (298.15 K) Ki	H-Bond interactions	Bond length (A˚)
1	zinc_3778874	-6.47	-0.29	18.0 uM	-	-
2	zinc_4950702	-6.96	-0.29	7.91 uM	ASP309:OD2	1.737
3	zinc_4950710	-6.75	-0.28	11.32 uM	-	-
4	zinc_5416379	-5.38	-0.28	113.76 uM	-	-
5	zinc_5416745	-5.56	-0.29	84.46 uM	-	-
6	zinc_5515709	-4.66	-0.36	380.88 uM	LEU372:O	1.921
7	zinc_5532983	-5.1	-0.31	181.87 uM	PRO429:O	2.015
8	zinc_8380994	-6.47	-0.27	18.16 uM	ARG435:O	2.11
9	zinc_15773472	-5.31	-0.	128.38 uM	SER314:O	2.025
10	zinc_15773474	-7.65	-0.21	2.47 uM	-	-
11	zinc_15773476	-8.43	-0.23	661.45 nM	-	-
12	zinc_15774214	-9.45	-0.25	118.1 nM	-	-
13	zinc_15774215	-8.96	-0.24	271.09 nM	ARG435:O	2.209
14	zinc_15774217	-9.93	-0.26	52.7 nM	LEU372:0	2.075
15	zinc_42906720	-5.42	-0.36	106.29 uM	LEU477:O	1.862
16	zinc_1404560	-6.47	-0.29	18.11 uM	LEU372:O	1.854
17	Letrozole	-10.09	-0.46	40.18 nM	-	-

Table.2. Docking result of some designed ligand as compared with prototype (Letrozole).

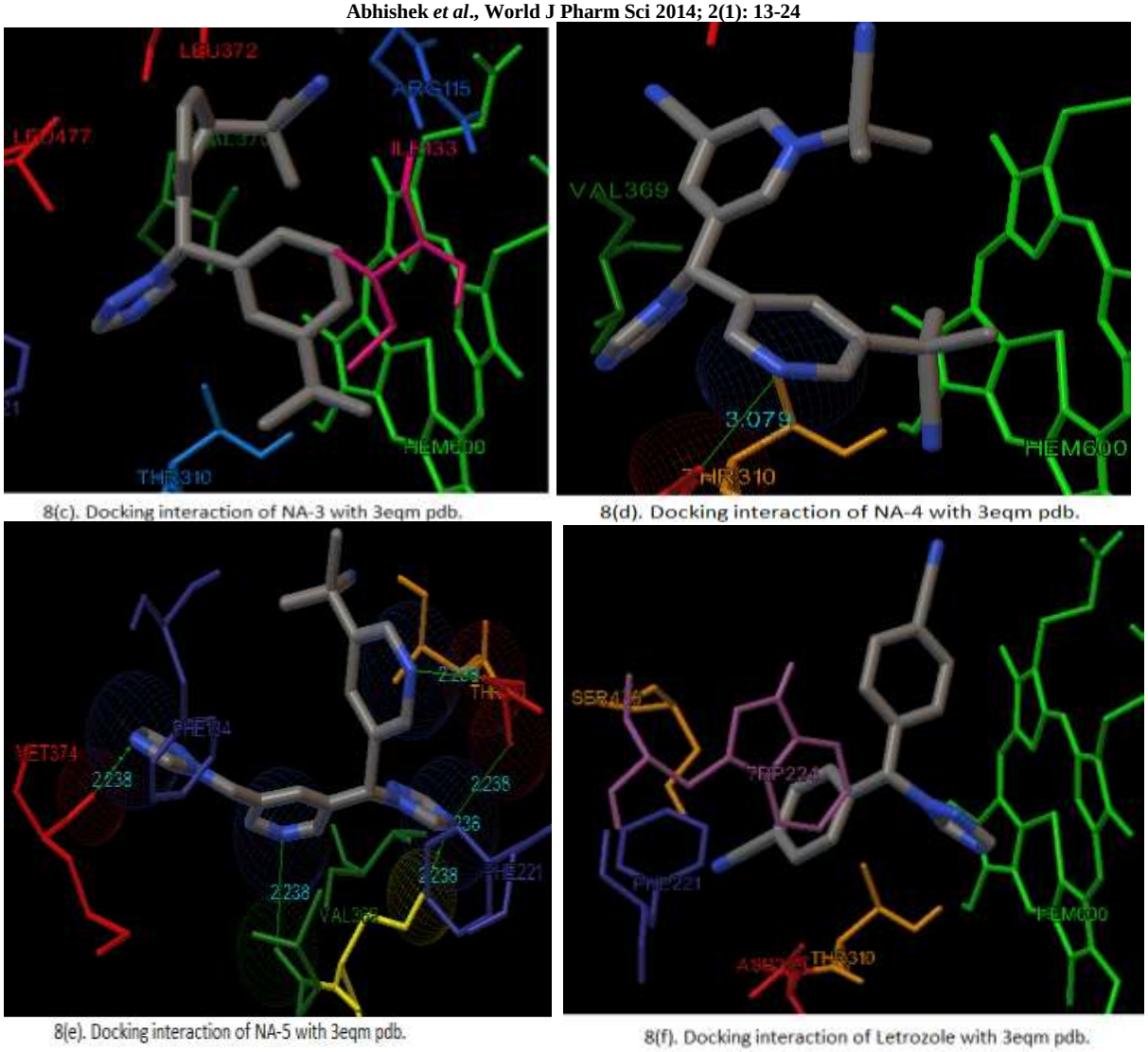
Sl.no:	Name of the Ligand	Binding energy (K.Cal/Mol)	Ligand efficiency	Inhibition constant (298.15 K) Ki	H-Bond interactions	Bond length (A˚)
1	NA-1	-11.6	-0.29	18.0 uM	-	-
2	NA-2	-11.02	-0.	7.92 uM	-	-
3	NA-3	-11.01	-0.28	11.32 uM	-	-
4	NA-4	-10.7	-0.28	113.76 uM	UNKO:N 1	3.097
5	NA-5	-10.66	-0.29	84.46 uM	MET374:NH	2.238
6	Letrozole	-10.09	-0.46	40.18 nM	-	-



8(a). Docking interaction of NA-1 with 3eqm pdb.



8(b). Docking interaction of NA-2 with 3eqm pdb.



8(c). Docking interaction of NA-3 with 3eqm pdb.

8(d). Docking interaction of NA-4 with 3eqm pdb.

8(e). Docking interaction of NA-5 with 3eqm pdb.

8(f). Docking interaction of Letrozole with 3eqm pdb.

Fig8. Docking interaction of all designed ligands (NA-1 to NA-5) respectively and prototype (Letrozole) with 3eqm pdb.

Table 3. Activity prediction of the designed ligands and Letrozole with PASS server*

Sl. No	Ligand name	Anti-fertility in female		Aromatase inhibitor		Anti-neoplastic		Anti-neoplastic (Breast cancer)	
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
1	NA-1	0,664	0,004	0,478	0,002	-	-	-	-
2	NA-2	0,695	0,003	0,128	0,014	0,548	0,057	-	-
3	NA-3	0,930	0,002	0,708	0,002	0,716	0,023	0,459	0,024
4	NA-4	0,830	0,002	0,606	0,003	0,712	0,012	0,334	0,046
5	NA-5	0,929	0,002	0,770	0,002	0,749	0,019	0,419	0,029
6	Letrozole	0,941	0,002	0,677	0,002	0,753	0,018	0,479	0,021

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

Table 4. admetSAR prediction of designed ligands and prototype Letrozole.

SI. no	Name of the Ligand	Blood Brain Barrier	Human Intestinal Absorption	$CaCo_2$ permiablity	AMES Test	Carcinogens	Rat-Acute Toxicity(LD50.mol/kg)
1	NA-1	0.9048	0.9970	0.5295	Non Ames Toxic	Non carcinogen	2.4677
2	NA-2	0.7847	0.9955	0.5058	Non Ames Toxic	Non carcinogen	2.8482
3	NA-3	0.9088	1.0000	0.5269	Non Ames Toxic	Non carcinogen	2.5770
4	NA-4	0.8368	1.0000	0.5171	Non Ames Toxic	Non carcinogen	2.6800
5	NA-5	0.9295	0.9907	0.5579	Non Ames Toxic	Non carcinogen	2.4751
6	Letrozole	0.9737	0.9965	0.6347	Non Ames Toxic	Non carcinogen	1.9916

Table 5. Lazar toxicity prediction of designed ligands and prototype Letrozole.

SI. no	Name of the Ligand	DSSTox Carcinogenic Potency DBS SingleCellCall	DSSTox Carcinogenic Potency DBS MultiCellCall	DSSTox Carcinogenic Potency DBS Mutagenicity
1	NA-1	Non-carcinogen	Non-carcinogen	Mutagenic
2	NA-2	Non-carcinogen	Non-carcinogen	Mutagenic
3	NA-3	Non-carcinogen	Non-carcinogen	Non-mutagenic
4	NA-4	Non-carcinogen	Non-carcinogen	Non-mutagenic
5	NA-5	Non-carcinogen	Non-carcinogen	Mutagenic
6	Letrozole	Carcinogen	Carcinogen	Non-mutagenic

Table 6. Drug Likeness properties of designed ligands and prototype Letrozole.

Sl.no:	Name of the Ligand	Drug Likeness Model Score	Molecular weight	Acceptor HB	Donor HB	Number of stereo centers
1	NA-1	0.92	421.18	8	2	2
2	NA-2	0.90	438.19	8	2	2
3	NA-3	0.03	371.21	4	0	2
4	NA-4	0.43	398.2	6	0	1
5	NA-5	0.45	385.15	7	0	1
6	Letrozole	0.85	285.10	4	0	0

Phase I Metabolic site prediction of Imatinib and its analogues: MetaPrint2D server (http://www-metaprint2d.ch.cam.ac.uk/) is used to predict Phase I Metabolic site of prototype and its deravitives. By setting the strictness of the fingerprint matching in "DEFAULT" and selecting model "ALL (Metabolite 2010.2)": As shown in (fig.9).

Results Color Scheme:

Red 0.66 <= NOR <= 1.00
Orange 0.33 <= NOR < 0.66
Green 0.15 <= NOR < 0.33
White 0.00 <= NOR < 0.15
Grey Little/no data

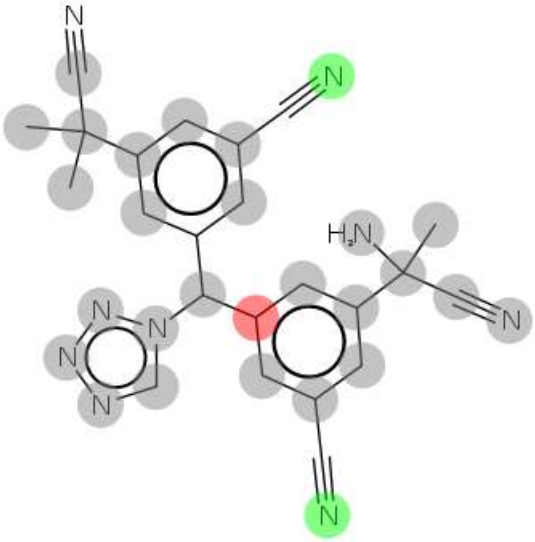


fig.9(a) Phase 1 metabolic site of NA-1.

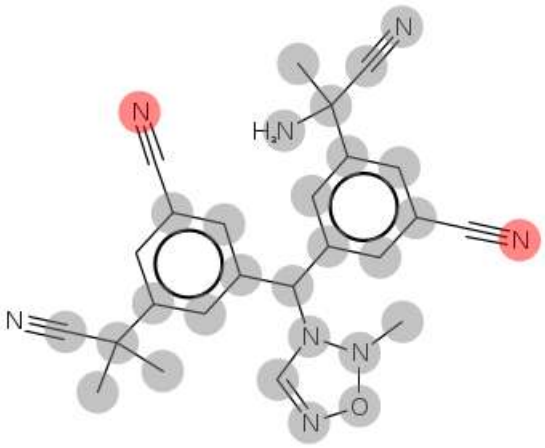


fig.9(b) Phase 1 metabolic site of NA-2

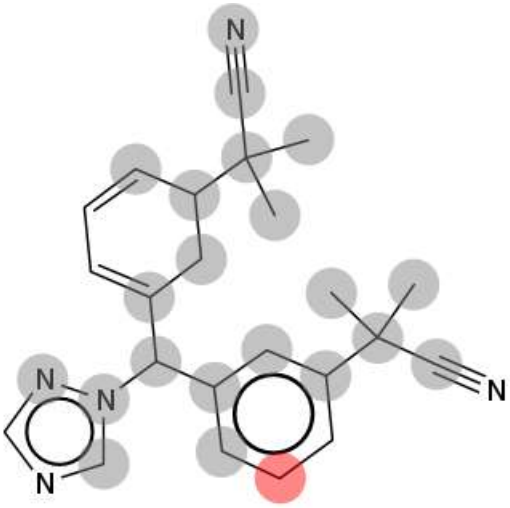


fig.9(c) Phase 1 metabolic site of NA-3

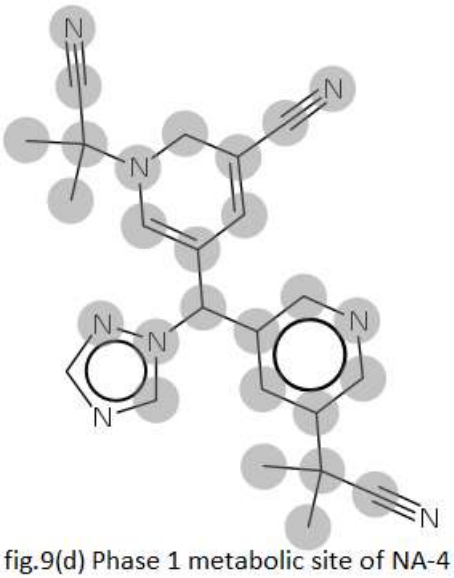


fig.9(d) Phase 1 metabolic site of NA-4

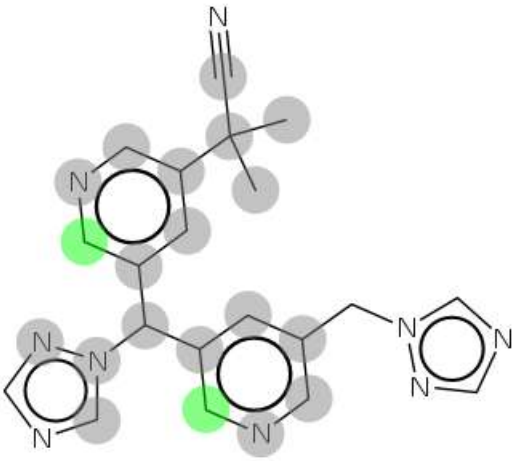


fig.9(e) Phase 1 metabolic site of NA-5

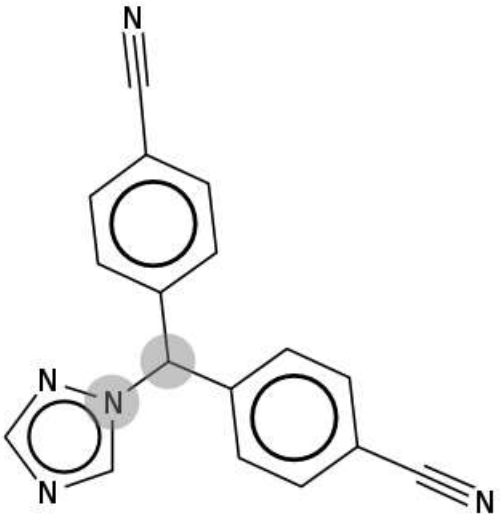


fig.9(f) Phase 1 metabolic site of Letrozole.

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