Original Article

Design, Docking, Insilco ADME Prediction Of Novel Indole Based Benzamide Scaffolds Targeting For Estrogen Receptor Alfa In Af-2 Domain For Effective Anticancer Treatment

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Abstract

Aim: To discover some novel indole based benzamide scaffold and their screening through in silico approach.

Background: Designed 7-substituted -1-(4-(piperidine-1-yl methoxy)benzyl)-1H-indole-3-carboxamide derivatives targeting on ERα modulators, several interactions between the ligand and amino acid residues that would probably elicit fruitful modulation of the receptor using 4XI3 pdb of ERα.

Objective: Studied in silico novel molecules of 7-substituted -1-(4-(piperidine-1-yl methoxy)benzyl)-1H-indole-3-carboxamide derivatives and test their abilities to modulate ER-α through human cell line cultures as anti-breast cancer agent.

Method: Designed novel 7-substituted -1-(4-(piperidine-1-yl methoxy) benzyl)-1H-indole-3-carboxamide derivatives and in silico method involved to study their virtual screening for the receptor modulation by molecular docking studies using Auto-dock Vina in PyRx. To determine the binding interactions for best-fit conformations in AF-2 binding site of the ERα receptor studied using Discovery studio visualizer (DSV) and ADME predictions by Swiss ADMET.

Result : The result based on the docking studies, The designed ligands B73bi, B73axiv B73bvi ,B73av, B73avi, B73avi, B73axiv, B74ai B74ai and B74bxiv have shown better Binding Affinity than rest, as compare with the standard drug Bazedoxifene (Baz). The observed result explained the presence of substitution at 7^{th} position of the benzamide on indole scaffold containing alkyl, ester, amide, N,N diamine groups shows promising interactions like BZD. Therefore, B73aiii carrying halide (G Score= -10.3), B73av carrying methoxy benzoate (G Score = -9.9), B73axiv carrying ethoxy (G Score= -9.4) were found to interact suitably with the active amino acid residues in the targeted cavity where reported interaction with the standard to be involved.

Conclusion: The most promising substituted benzamide analogue on indole can be synthesized and evaluated to verify the ani-cancer activity for breast cancer.

Keywords: Docking, Breast cancer, Indole scaffold, Benzamide, Bezedoxifen ER α, Discovery studio visualizer, PyRx, Auto dock Vina SERMs, Swiss ADMET.

1.0 INTRODUCTION

Breast cancer (BC) occurs in worldwide in women of each country at any age after puberty but with rising rates in future life. Breast carcinoma in human is the second leading reason for mortality among women. In 2020, there were 2.3 million women diagnosed with BC and number of death 685 000 globally. As of the end of 2020, there were 7.8 million women active cases of BC in the past 5 years, Around 50 % of BC develop in women due to BC risk factors rather than gender like female or age above 40 years. Such factors increase the risk of BC including obesity, radiation exposure, excessive intake of alcohol and tobacco , reproductive disorder and family history of BC etc. [1,2] Estrogen and the estrogen receptor (ER) are recognized to be protruding drivers of breast tumorigenesis and breast cancer progression The first line treatment of BC used hormonal therapy in the estrogen sensitive BC. [3-9] ER-α is responsible for regulating the record of atomic DNA that is viewed as a significant part of breast malignant growth signal generation gives a book biomarker of BC.[10] Clinical treatment has been using drugs acting on ER-α called as SERMs (Selective Estrogen Receptor Modulators). SERMs are prepared for contending with endogenous estrogens to adjust the movement of estrogen receptors [11]. Ligand shows mechanism of action of ER-α mediated by at least two separate activation functions (AFs), AF-1 in the N terminus, and AF-2 in the ligand based domain (LBD). The AF-1 activity is controlled by growth factors acting through the MAP kinase pathway while AF-2 activity is responsive to ligand binding on ER-α. The binding of agonist's ligand activates AF-2 activity while the binding of antagonists does not Recent structural studies suggest that ligands regulate AF-2 activity by directly affecting the structure of the LBD. Conformational change involving the repositioning of helix-12 , present on C-terminal of the LBD is essential for AF-2 activity.[12] SERMs bind to the ER and they may act as receptor antagonists or mixed agonists/antagonists by modulating receptor conformation and modifiable co-activators [13-15] Different types of SERMs that have been utilized in first and second line clinical treatment includes Tamoxifen and Raloxifen that demonstration straightforwardly as ER-α resistance and undesirable side effect on uterine, bone, and other tissues. [16]. Bazedoxifene was used advanced SERM developed by the changing essential pharmacophoric features of raloxifene, by substituting the benzothiophene core of raloxifene by indole [17,18]. Recently, bazedoxifene in have been used for treatment of metastatic BC at stage IV [19-21].Present work based on ligand based drug design. LBDD denotes to pharmacophore, quantitative structure-activity relationship (QSAR) using computer aided drug design. Hence, based on the availability of the known the information of the ligands and target receptor structure, we should apply proper method to build virtual screening model. In addition, assimilating the different methods together is helpful for making up their deficiency and improving the reliability [22] To discover new drugs entities, has become powerful tool in drug discovery research approach wherein the number of methods and software used to study interaction of ligand/target approach [23] The current search zeroed in silico examination of 7 substituted Benzamide at indole is as a scaffold for research. In silico screening process carried out by analyzing large compound libraries computationally. The steps comprise protein structure preparation, ligands database preparation and docking calculations. The energy function that evaluates the binding free energy between protein and ligand is reflected as a scoring function .[24,25].To study protein surface atoms, site points, different types of interactions with their bond types of various possible confermers such data are internally available in the docking software like in PyRx, Schrodinger-Maestro 11 or Discovery Studio.[26] The best conformer with energy minimized ligands are predicted by computational approaches that 'dock' library of ligands on to the structures of target proteins and 'score' their potential complementarities to binding sites.[27] Docking studies over ERα active warrant thorough analysis of the target site in the protein so as to facilitate design of probable ligands. ERα has five domains in the whole protein with distinct functions. Domain A/B (N-terminal) consists of activation function 1 (AF-1) which contributes to the transcriptional activity of ER by undergoing conformational change in response to activation by estrogen. This is the same site which is also essential for interaction with co-regulators [28, 29] The crystal structure of the homo dimer Estrogen receptor 4Xi3, represents a human estrogen receptor-ligand-binding domain in complex with Baz provides a suitable guiding template for studying the binding interactions of designed ligands within the AF-2 cavity where interactions can be viewed up to the proximity

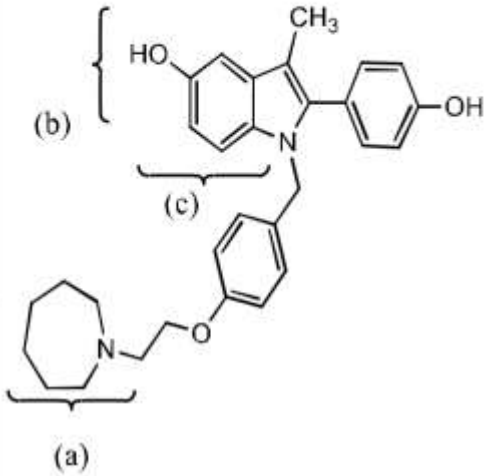
of 0.02 Å [30]. For molecular docking PyRx software PyRx (Virtual screening tool) is used and 3 dimensional interactions of ligand receptor complex after docking was studied by using Biovia Discovery Studio visualizer. Docking result where compared with marketed drug Baz. [31,32] The same crystal structure has been used in efforts to generate Benzamide derivatives substituted at 7 positions as ERα modulator. Docking studies over ERα active permit thorough analysis of the target site in the protein so as to help design of probable ligands. ERα has five domains in the whole protein with distinct functions. Domain A/B (N-terminal) consists of activation function 1 (AF-1) which contributes to the transcriptional activity of ER by undergoing conformational change in response to activation by estrogen. This is the same site which is also essential for interaction with co-regulators [33, 34]. The domain C encodes a centrally located DNA-binding domain (DBD) which is essential for sequence specific binding of ER to DNA and this regulates the expression of target gene [35, 36]. The C/D- domain, a hinge region includes amino acid sequence that stimulates nuclear localization signaling and facilitates post-translational modification of ER resulting in the activation of ER signaling to cells. Finally, E/F domain, the ligand-dependent activation function 2 (AF-2), located in the C- terminal contains a ligand-binding domain that also serves as site for interaction with co-regulator proteins [37, 38]. Hence, the domain E/F provides required site for interaction with the ligands. Moreover, the AF-2 being conserved only in ERα, provides the required specificity for anti-estrogenic activity in the cancerous tissues, especially in breast cancer, as mRNA transcripts of ERα have been prominently expressed in immortalized human breast cancer tissues [39 40]. Reported work proposed that is an attempt to fulfill designed and evaluated Reported work proposed that is an attempt to fulfill designed and evaluated novel analogues of 7 substituted benzamide on indole for further lead optimization and in silico study to identify more potent Analogues of anticancer for the breast cancer.

Design: Bazedoxifene (1H-indo-5-ol,1-[[4-[2(hexahydro-1H-azepin-1-yl) ethoxy] methyl]2-(-4-hydroxyphenlyl)-3-methyl; acetic acid) is third generation SERM is one of the compound comprised with indole system has served as a core unit in SERMs, and when an amine is attached to the indole with a benzyloxyethyl, the resultant compounds were shown to have no preclinical uterine activity while sparing rat bone with full efficacy at low doses[41] This novel indole derivative provided a first-hand scaffold to work-on and prepare congeners that would have similar, if not identical, binding properties in AF2 domain, and modulate the transcriptional effects of ERα.

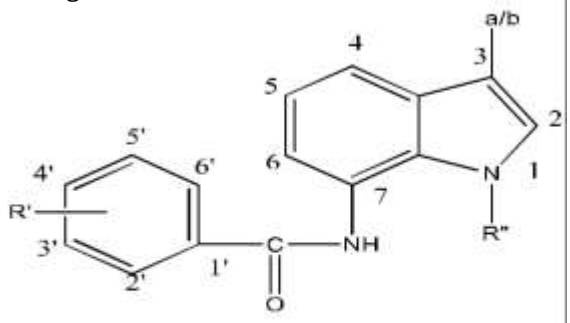
Important amino acid residues (AAR), that have been comprehensively studied and reported to constitute partly the AF-2 domain of ERα, are -Thr347, Asp351, Glu353, Arg394, and Phe404. It has been reported [42] that there occurs a salt-bridge interaction between anionic side chain of Asp351, Thr347 and the cationic nitrogen of the alkylazepane part of Baz (a). The phenolic hydroxyl group in Baz (b) forms a tripod sandwiching a water molecule with the guanidine nitrogen of Arg394, and the peptide linkage between Leu387, Met388 , and Glu353 , involving hydrogen bond interactions amongst them. There is a π -π stacking interaction between indole nucleus (c) and phenyl ring in the side chain of Phe404. The 2-(4-hydroxy) phenyl substitution on the ligand is not shown to interact with any specific amino acid residue on the protein, however the extended structure seems to be sandwiched between two strands of the protein densely populated with Ile and Leu residues , and suggest possibilities of hydrophobic interactions between them. Apart from these, the amino acid residues in the vicinity of those reported previously such as Leu387, Ile424, Leu428, Leu354, Asp 351, Leu 391, Leu 428,Trp 383.Ile 424,Leu 525 and Leu539 shows hydrophobic interactions, Asp 351, , Leu 391,Leu 428,Trp 383.Ile 424,Leu 525 and Try383, Met343, Thr347, Thr425 in polar interactions can be observed for probable interactions with appropriate and complimentary structural features in the new entities of novel indole based benzamide.

Table no 1 Standard Bazedoxifene and Design Ligand of 7 substituted Benzamide analogs of Indole

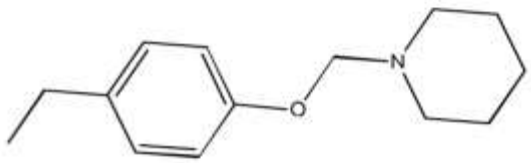
Bazedoxifene



Design Ligand of 7 substituted Benzamide analogs of Indole

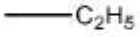
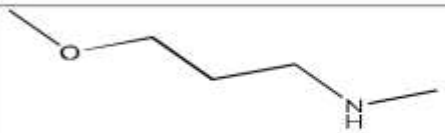
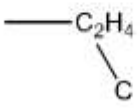
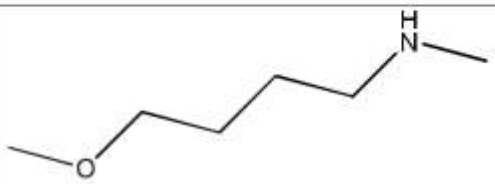
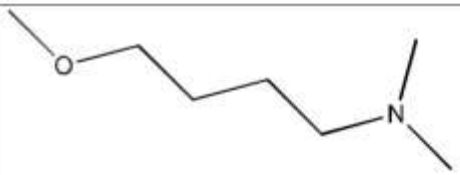
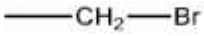
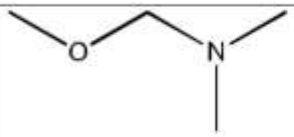
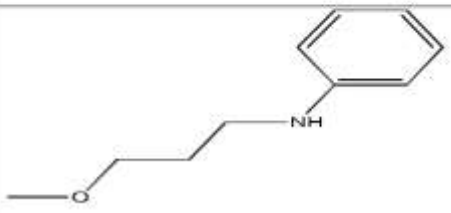


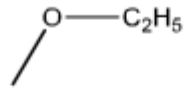
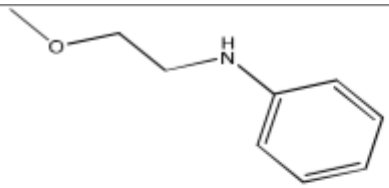
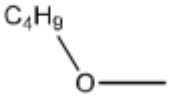
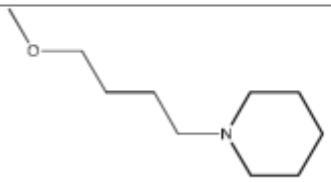
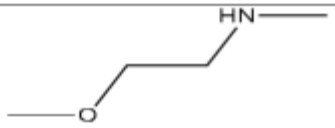
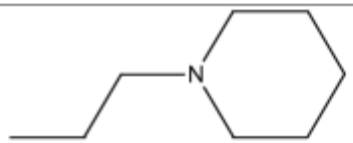
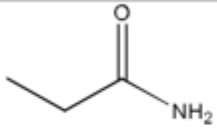
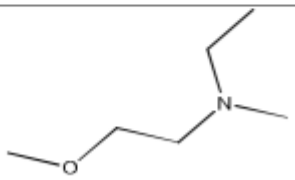
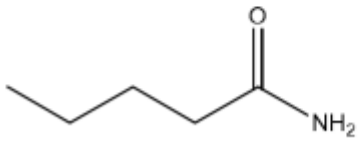
R"-



a- Amide group
b- Hydroxyl group

R'- (3' and 4' position)

i		**Xi**	
ii		**Xii**	
iii		**Xiii**	
iv		**Xiv**	
v		**Xv**	

vi		**Xvi**	
vii		**Xvii**	
viii		**Xiii**	
ix		**Xix**	
X		**XX**	

2.0 MATERIAL AND METHODS

2.1 Preparations of analogues for Docking-

The basic structures of Standard anticancer agent Bazedoxifene is (shown in table 1) The structures were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Eighty Novel indole based benzamide were designed (shown in table 1) and draw busing Chemdraw altra 12 softwere were converted to SDF files. Open Babel tool was used for optimizing designed analogues with force fields MMFF94 and GHEMICAL. Analogues energy was minimized by Conjugate Gradient and Steepest Descent optimization algorithms. The cycle consisted of 50000 steps with convergence criteria of 0.001 kcal/mol/Angstrom. Finally all ligand option was used to convert the minimized files to PDBQT format to generate their atomic coordinates are used as input in PyRx software (http://PyRx.sourceforge.net/)[42]

2.2 Selection of 4Xi3 protein and optimization-

A search for Estrogen Receptor alpha (ER-α) structure in Protein Data Bank (PDB) [www.rcsb.org/pdb] revealed several hits with bound ligands and drugs. In general, the selection of the receptor is based on highest possible resolution, no mutations or modified residues and the presence of bound ligand or drug [43] in particular. The resolution ensures that 3D structures utilized for docking were of a good quality and on the other hand, the structure should be devoid of any mutations, this is because mutations might have profound effects on the final confirmation of a protein Moreover, a co-crystallized bound ligand represents better geometric orientation within the active site space of the protein. Therefore, the 3D structure of ERα bound with selective estrogen receptor modulator i.e. Bazedoxifen (PDB ID: 4Xi3), was selected as the preferred docking target protein.[44] and was analyzed for its active site by discovery studio visualizer (http://accelrys.com).[45,46,47] Preparation of the proteins was carried out with the Biovia Discovery Studio Visualizer (DSV) 2017 (Prepare protein protocol).

After downloading the 3D structures of protein molecules from the PDB database, preparation of protein involved adding hydrogen atoms, defining the bond orders, deleting unwanted water molecules and salts followed by optimizing hydrogen bond network. Protein functions as a monomer, remove other protein chains that are present in the asymmetric unit of the PDB file except a chain which contain standard interaction with Bazedoxifene. Add Polar hydrogens and optimize the hydrogen-bonding network. The proteins were energetically minimized and active sites were predicted with the selection of maximized GRID parameters using DSV. Finally, save the prepared structure of protein in PDB file format for docking. Details of the selected pdb of cocrystalized ER alpha with BZA given in table no 2.

Table 2. The details of ER alpha using (PDB ID-4Xi3)

Pdb id	4Xi3
Title	Estrogen Receptor Alpha Ligand Binding Domain in Complex with Bazedoxifene
DOI	10.2210/pdb4XI3/pdb
Resolution	2.49 Å
Organism	Homosapiens
Expression system	Escherichia coli BL21(DE3)
Method	X-Ray Diffraction

2.3. Identification of Cavity and Active Amino Acid Residues-

The crystal structure of the homodimer Estrogen receptor -4Xi3, represents a human estrogen receptor-ligand-binding domain in complex with Bazedoxifene , and provided a suitable guiding template for studying the binding interactions of designed ligands within the AF-2 cavity where interactions can be viewed upto the proximity of 2.60 Å. Molecular modeling study were carried out in an attempt to understand the molecular basis of interaction of compounds 73a-73b(I to xx) and74a-74b(I to xx) with RE-α. As the rigid docking protocol possesses some pros and cons as it does not take account of the protein flexibility which has a significant impact on the binding modes and the docking protocol [48].

Import both designed ligands with standard BZD and prepared protein in Auto dock Vina Wizard and converted both macro molecules into PDBQT format. The three dimensional grid box (size_x = 48.93; size_y = 46.57; size_z = 53.04) was generated automatically. The active amino acid residues were selected to define the cavity with the help of the Toggle Selection Sphere option The grid box was aligned so as to occupy all the active binding sites and essential residues [49,50].

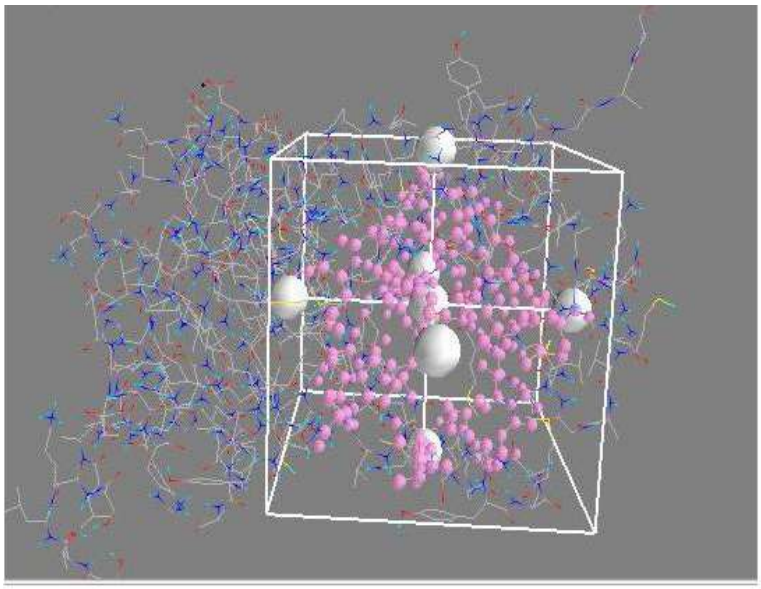


Figure 1: Grid generation for protein 4Xi3 and Novel indole based benzamide.

2.4. Docking Procedure

Molecular docking of analogs and 4Xi3 were subjected to docking using uto dock Vina Wizard of PyRx virtual screening 0.8 version software. A study of non-bonded, non-covalent interactions between a receptor or active site region of a protein and a drug or chemical molecule forming an intermolecular complex was predicted by docking.. Macromolecule and ligand files are then opened in this program. Macromolecule and ligand files in .pdb format are then converted into .pdbqt format via the PyRx assisted Vina AutoDock program. The .pdb format in the ligand and receptor shows no charge on the molecule, whereas the .pdbqt format shows a partial charge on each atom [50].Then tethered using a Grid box determined by the position of the crystallographic ligand on the alpha estrogen receptor binding site at the validation stage. The result of this tethering will result in the affinity binding value of each tested ligand. Docking scores (kcal/mol/Angstrom) obtained from PyRx software along with interaction parameters like binding affinity (Docking score), RMSD (upper limit), RMSD (lower limit).The PyRx software uses a measure of distance between the experimental and predicted structures to compare the accuracy of the predictions. Root mean square deviations are calculated relative to the best mode and using only movable heavy atoms. Two variants of RMSD are lb-lower bound and ub-upper bound, differing in how the atoms are matched in the distance calculation.[51] The parameters used are the lowest affinity binding values of each ligand which will proceed to the next stage of conformational visualization. Docking of Nine conformations of ligands by default to a receptor followed by evaluation of the molecules with respect to the geometrical orientation and complementarity with respective of shape, size, and electrostatic, hydrophobic properties. The best interactions were visualized by Discovery Studio visualizer. [52-53]. The conformational visualization is performed on the Biovia Discovery Visualizer software by opening the receptor structure as a macromolecule which is then tethered with each of the lowest energy ligands to see the conformation and interaction with the amino acid residues formed. Induced fit docking protocol is a ground-breaking protocol which includes protein flexibility and at the same time reducing the computational time without compromising with the accuracy in the prediction of favorable binding mode and conformation of ligand at the receptor site. The final result of docking includes affinity prediction (scoring) for the molecules investigated, yielding a relative rank ordering of the docked compounds with respect to affinity, reported as kcal/mol. Higher negative binding energy indicates higher binding affinity. Higher docked scored complexes were evaluated for the analysis of binding interactions using DSV.

3. RESULT

All the designed analogues in the targeted cavity were successfully docked on 4Xi3. Structure of the designed molecule, molecular formula, Lipinski's rule of five, binding affinity (kcal/mol), and active amino acid residues are presented in Table 3. Docking poses of higher binding affinity analogues in 3D- and 2D-images along with the number of hydrogen bonds involved in the interaction are shown in Table 4.

3D and 2D image of docked structure of bazedoxifene in 4XI3 with 0.02 Å rmsd shown in figure 2 (A and B). Similarly top scored designed analogues interactions with protein are shown in Figure 3 (A to F) .The interactions of functional groups on ligands that interact with active amino acid residue in the targeted cavity are focused in the images. All the designed analoges were checked for the violation of Lipinski's rule of five along with binding interaction studies for the optimization of the molecule. The properties of the derivatives for obeying the rule were determined from Quick Prop panel. These functionalities having potentiate positive response for ER modulation which will be synthesized and tested for their activity to bind and modulate the receptor in an in vitro assays, which becomes further aspects of research.

Table 3. Properties, Lipinski's Rule of Five, binding affinity, and active amino acid residues

Name of Compd.	Molecular Formula	Lipinski's Rule of Five		Binding affinity (kcal/mol)	Active amino acid residues (distance in Å)
B73aiii	C30H31ClN4O3	Molecular weight (<500DA)	531.05 g/mo	-10	H with Thr347[O]:2.22, O with Leu525[H]:2.45, O with Leu346[H]:2.84, π-π stacking ASP351 [O]:4.84
		XLogP(<5)	4.65		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	4		
B73av	C33H39N5O4	Molecular weight (<500DA)	569.69	-9.9	H with SER536[O]:1.64, H with SER536[O]:2.57, H with Leu346[O]:2.11, π-π stacking ASP351 [O]:3.78
		XLogP(<5)	4.01		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	6		
B73axiv	C33H39N5O4	Molecular weight (<500DA)	569.69	-9.4	H with Lys 529 [O]:2.35, H with Asp (351) [C]:3.73, π-π stacking electron ASP351 [O]:3.87
		XLogP(<5)	4.27		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	6		
B73bxiv	C32H38N4O4	Molecular weight (<500DA)	542.67	-9.1	H with Arg353[O]:2.32, H with Leu346[O]:2.11, C with Glu 353[H]:342 π-π stacking with Trp 393 : 5.55
		XLogP(<5)	4.78		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	6		
B74ai	C33H38N4O3	Molecular weight (<500DA)	538.68	-9.0	O with LYS 529 [H]:2.58, H with THR 347 [O]:2.15, π-π stacking ASP351 [O]:3.78
		XLogP(<5)	4.02		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	4		
B73aviii	C33H39N5O4	Molecular weight (<500DA)	569.69	**-9.0**	O with LYS 529 [H]:2.21, H with THR 347 [O]:2.06, H with ASP 351 [O]:2.16,
		XLogP(<5)	3.4		
		H-Bond donor (5)	3		

		H-bond acceptor (<10)	6		π-π stacking ASP351 [O]:3.70
B73axx	C34H39N5O4	Molecular weight (<500DA)	581.70	-8.9	O with LYS 531 [H]:1.95, H with ASP 351 [O]:3.49, H with ALA 350 [O]:2.2402, π-π stacking ASP351 [O]:3.23 and 3.76
		XLogP(<5)	3.63		
		H-Bond donor (5)	3		
		H-bond acceptor (<10)	5		
B74bix	C33H40N4O4	Molecular weight (<500DA)	556.70	-8.8	O with LYS 531 [H]:2.59, H with ALA 350 [O]:2.81, C with Leu 391[H] : 4.89 π-π stacking ASP351 [O]:3.79 and 3.21
		XLogP(<5)	4.03		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	6		
B74axvii		Molecular weight (<500DA)	651	-8.7	O with ILE 326[H]:2.31, H with PRO324 [O]:3.58, O with ARG 394 [H]:2.38, C with Glu 358 [H]:3.73&3.32 π-π stacking ASP351 [O]:3.3 and 3.9
		XLogP(<5)	4.43		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	6		
B73axvii		Molecular weight (<500DA)	651	-8.6	H with Ala 350 [O]:2.04, O with Asn 532 [O]:2.41, H with LYS 531 [H]:2.17, π-π stacking (5.07 & 5.29).
		XLogP(<5)	3.22		
		H-Bond donor (5)	2		
		H-bond acceptor (<10)	6		

The figure 1A to 1 D shows superimposition of the docked 3d and 2d structure of best conformation of BZA and protein structure of 4xi3 complex summarizes the orientation of the native ligand in the active site, the constant interactions with the key amino acids, Electronic interactions, Hydrobhobic interactions hydrogen interactions etc. The simulation results of molecular tethering were analyzed for conformation and interaction using Biovia Discovery Studio Visualizer software taking into account the types of interactions and amino acid residues involved in the 4Xi3 and design entities complex with various conformer generated during docking by Auto dock vina wizard.3D and 2D Poses of selective best induced fit analogues with 4Xi3 crystalize structure of the ER-alpha receptor mentioned in the table no 4

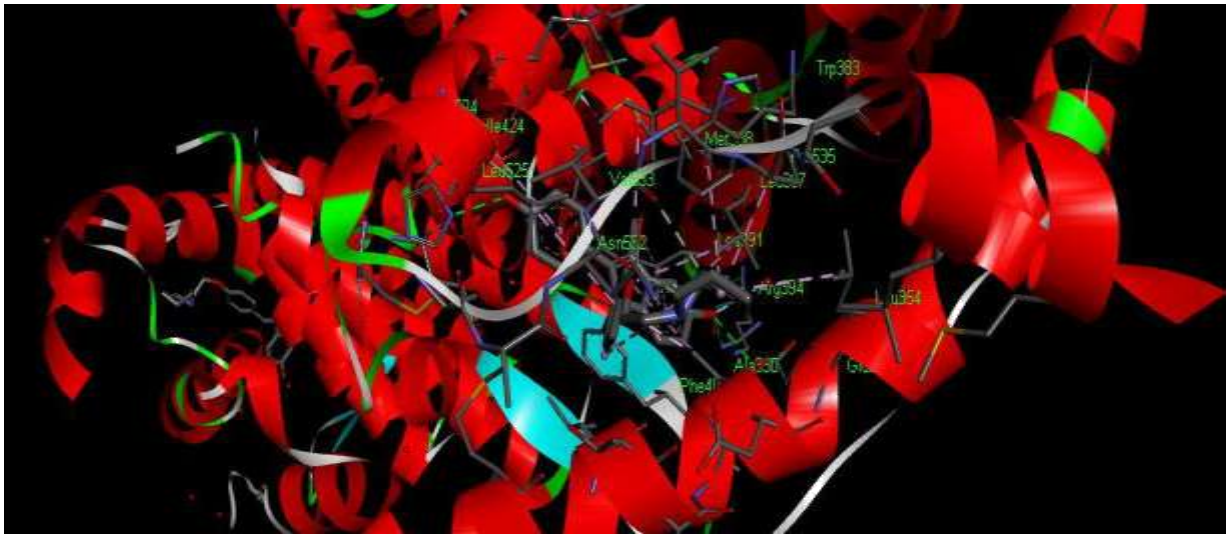


Fig 1A: 3D structure of Superposition of the docked structure

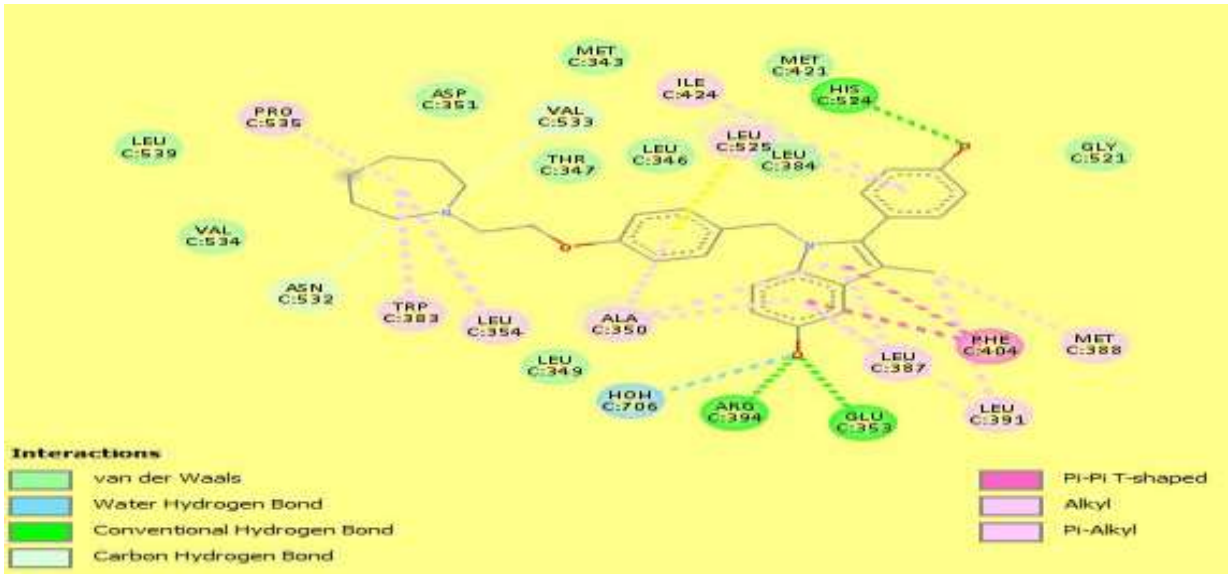


Fig 1B: 2D structure of Superposition of the docked structure of bazedoxifene in 4XI3 with 0.02 Å rmsd.

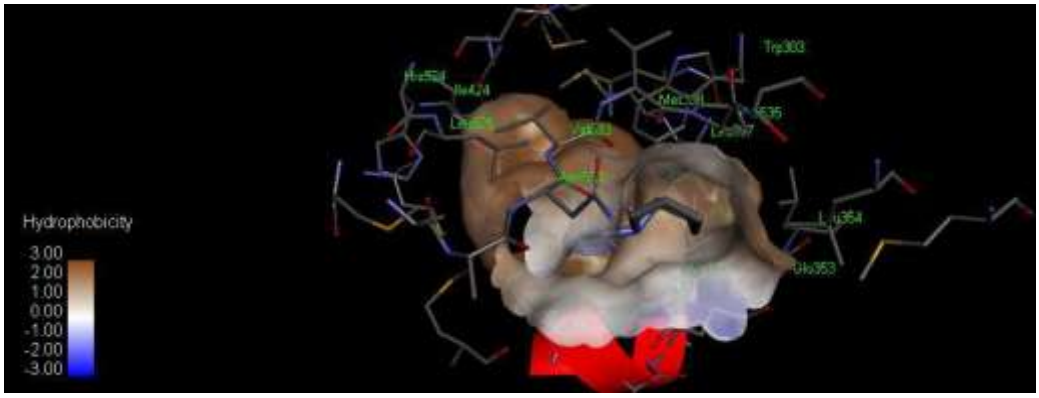
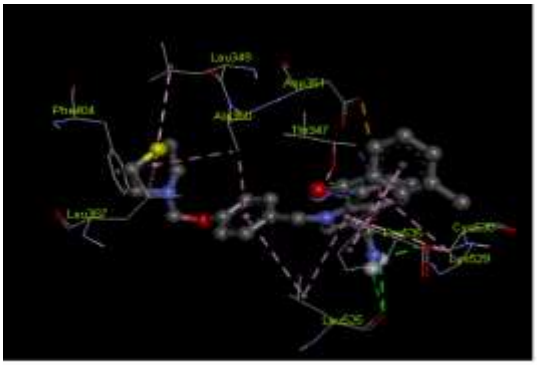
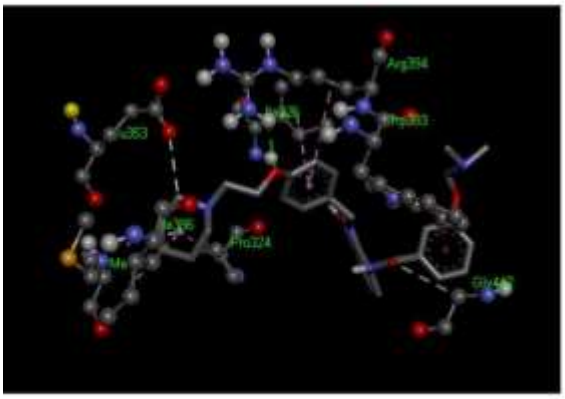
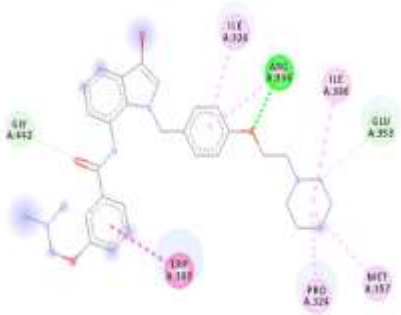
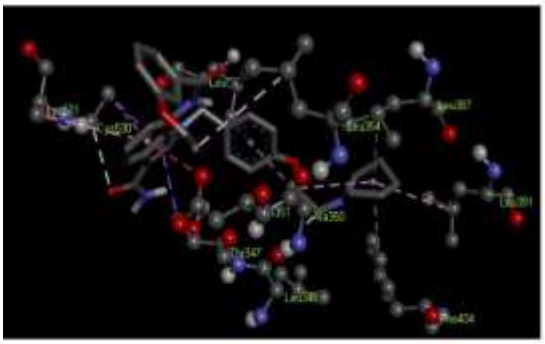
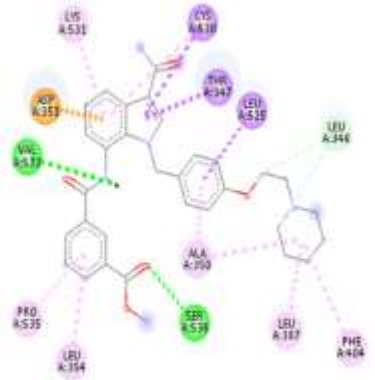
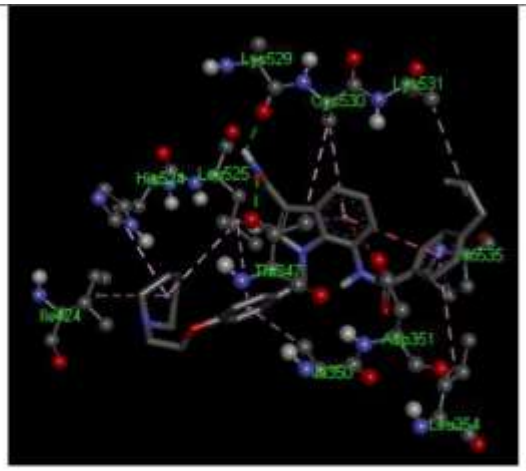
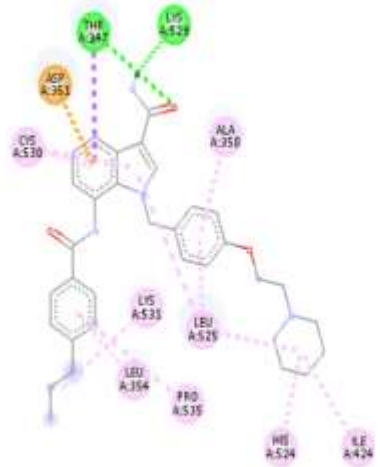
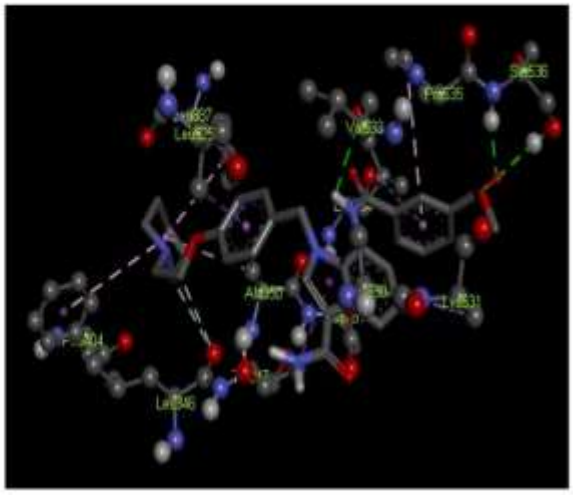
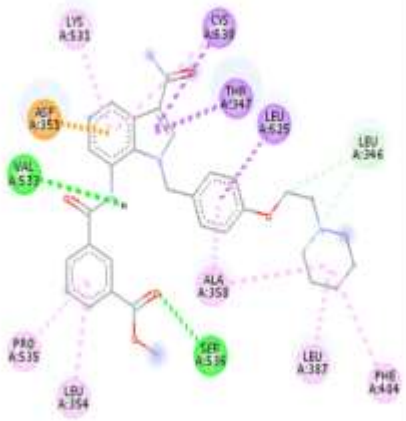
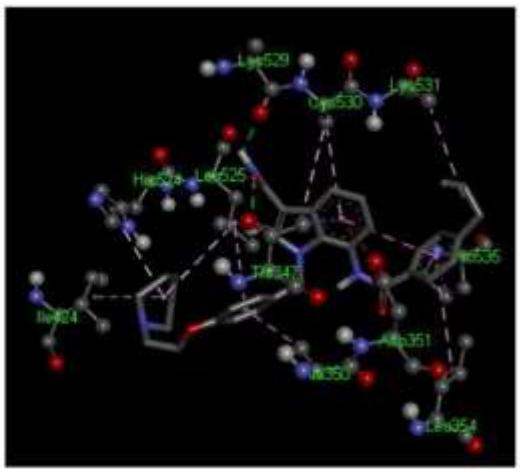
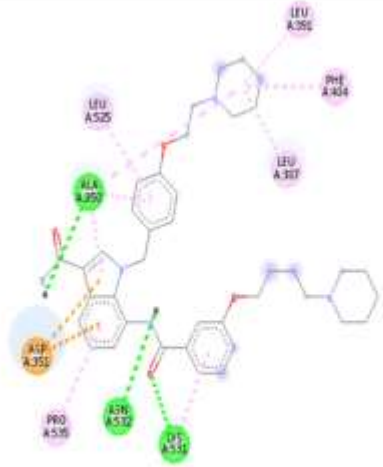
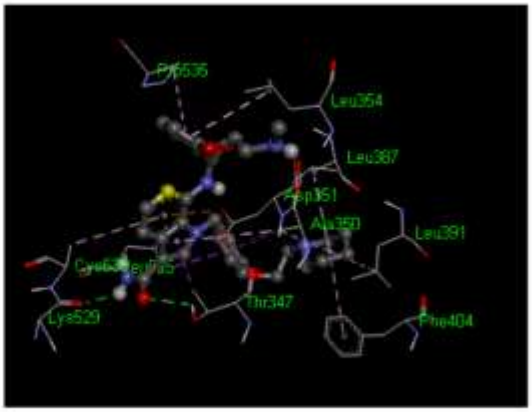
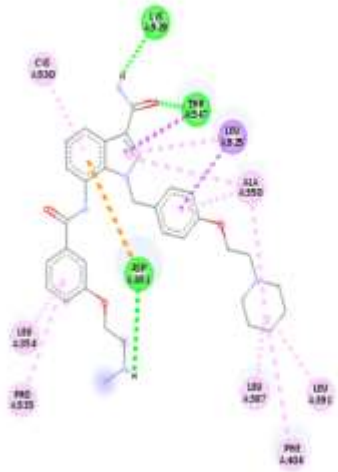
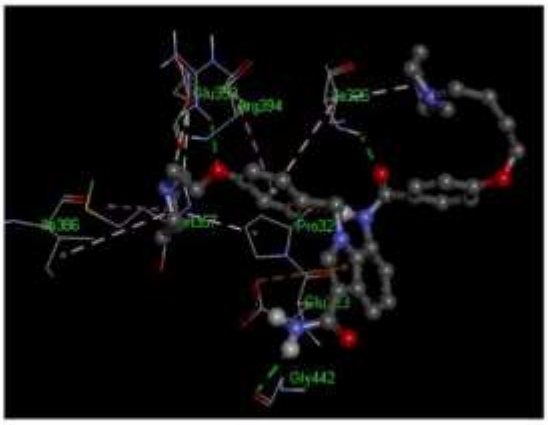
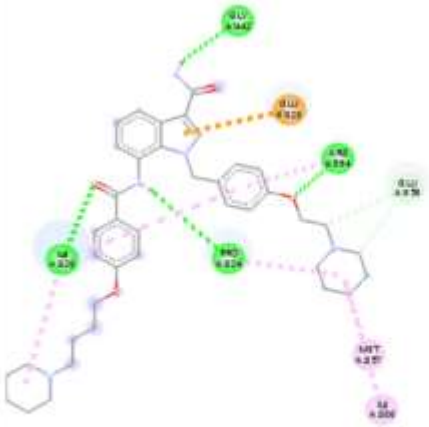
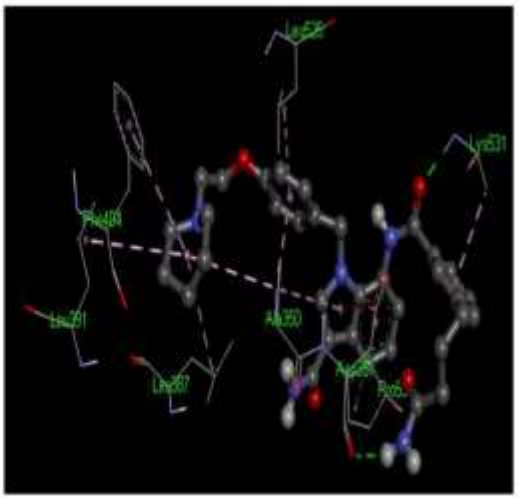


Fig 1C : Hydrophobic interactions

Table 4. 3D- and 2D-images of docking poses along with number of hydrogen bonds involved.

Name of Compd.	3D-docking pose	2D- docking pose	No. of hydrogen bonds involved
B73aiii (-10)			**3**
B73bxiv (-9.1)			**1**
B73axiv (-9.4)			**2**
B74ai (-9)			**2**

B73av			**2**
(-9.9) B73axvii			**3**
(-8.6)			

B73aviii **(-9)**			**3**
B74axvii (-8.7)			**4**
B73axx **(-8.9)**			**3**

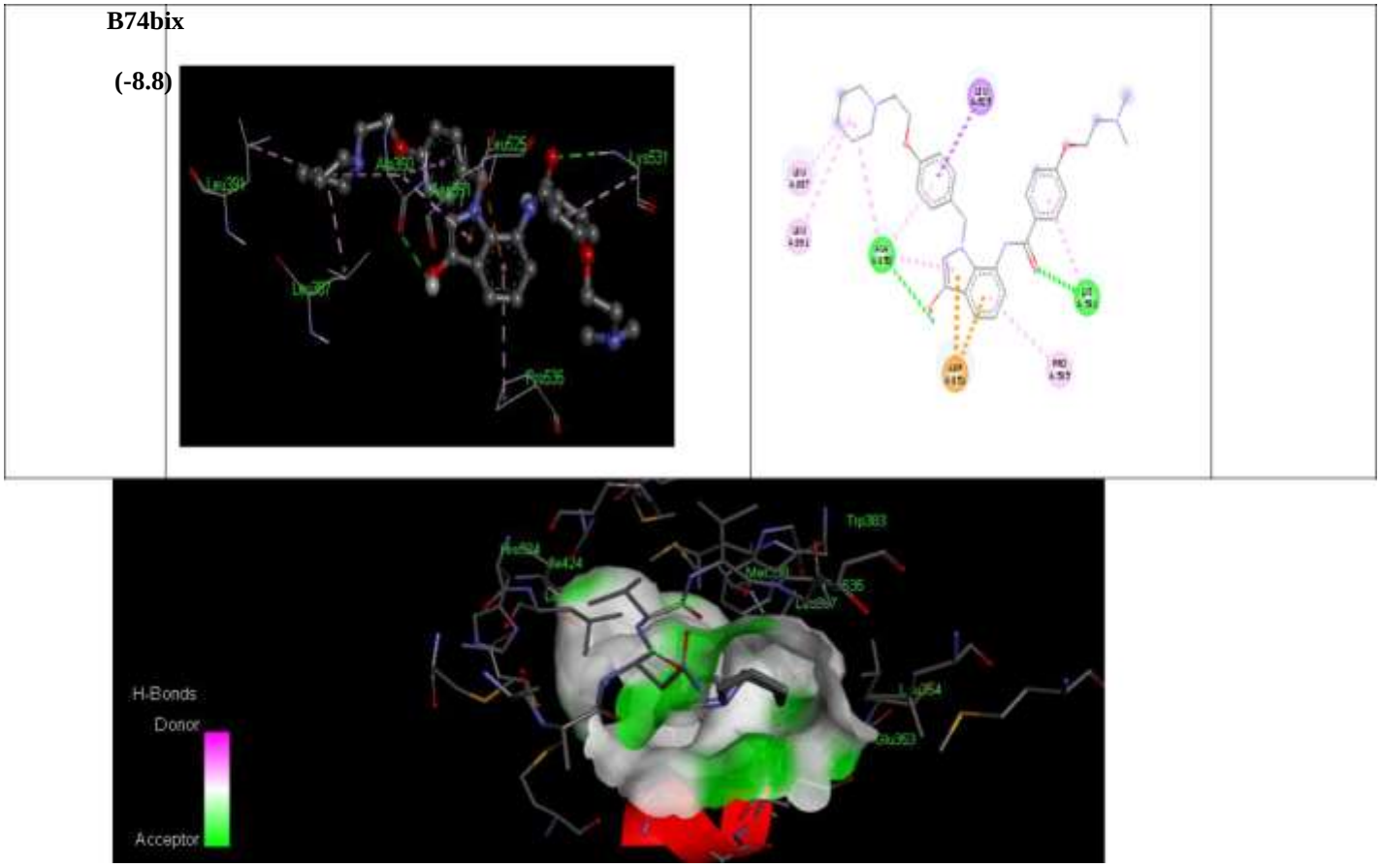


Fig 1D: Hydrogen bond interactions

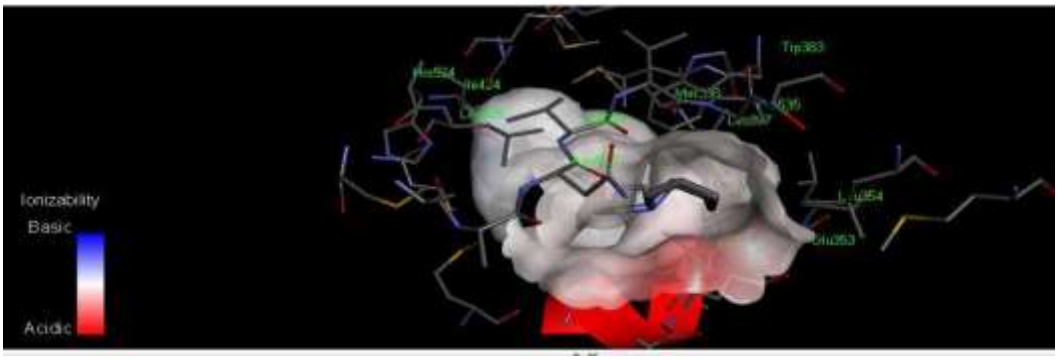


Fig 1E: Ionization associated with interactions

4.0 DISCUSSION

The data for parent structure of Bazedoxifene and its novel designed Benzamide analogues of indole (B73a-B73b and B74a-B74b) with functional groups substitution is mentioned in table 1. All the designed analogues in the targeted cavity were successfully docked on 4Xi3. The resultant outcome of the analysis in terms of type of interactions, active amino acid residue involved in the interactions, inter-atomic distances, binding affinity (kcal/mol) mentioned in Table 3.Analysis of ER α active site was carried out for 4Xi3 by discovery studio Visualizer software is depicted in figure 1(A and B) where amino acid residues Arg 354,Glu 353, His 524 shows conventional Hydrogen bonding and Asn 532,Trp 383,Lue 354,Ala 350,Leu 387,Leu 391,,Leu384,Leu 525,Ile 424, Asp 351,val533,Trp 347,Pro 535 shows van-der waals, carbon hydrogen bond pi-alkyl, alkyl-alkyl, Pi stack interactions with the known standard drug Bazedoxifene. The newly designed analogues of Benzamide from table 1 were then docked at the same active site of ER α to predict best analogues binding mode. [54] Top scored Benzamide analogues interactions with protein ID: 4Xi3 are shown in table no 3.

Discussion in present docking analysis, datasets of 80 analogues each Benzamide analogues were docked at the active site of ER α protein using PyRx software. The docking data of ligand-protein molecular interactions

of best 10 analogues from B73a-B73b and B74a-B74b analogues namely B73aiii , B73bxiv, B73axiv B74ai, B73av, B73axvii, B73aviii , B74axvii , B73axx and B74bix are depicted in table no 4 respectively. It becomes evident that the best analogues are in the acceptable range by Lipinski rule of 5 [55] shown in table 2 and may have similar pharmacological properties like parent structures of Bazedoxifene as ER α modulators. It is hoped that some of them may possess better anticancer activity for the treatment of breast. From above discussion related to usefulness of docking studies, using PyRx software, has predicted and confirmed that some novel analogues of 7 substituted Benzamide on indole Stafford which show similar binding scores as compared parent structure of Bazedoxifene. These analogues do show favorable drug likeliness properties and bind to the same active site of target protein where drugs like Bazedoxifene are known to bind.

CONCLUSION

This study performed with PyRx software strongly supports the importance of computational approach in early part of drug discovery research. It results in saving enormous amount of time, resources and money. The 7-substituted -1-(4-(piperidin-1-ylmethoxy)benzyl)-1H-indole-3-carboxamide derivatives were designed to have a potential for modulating ERα. Amongst the designed derivatives, ligands that have shown binding energies (between -10.008 to -8.6 kcal/mol) comparable with the binding energy of the standard, BZA, are reported here. From observations presented in Table no. 3, we conclude that amongst all the designed ligands, ligand B73aiii , B73bxiv, B73axiv B74ai, B73av, B73axvii, B73aviii , B74axvii , B73axx and B74bix have promised potential to bind ERα receptor effectively. The novel 7 substituted Benzamide on indole Stafford analogues follow Lipinski rule of 5. Based on the results of the analysis of silico designing including compounds that act as Selective Estrogen Receptor Modulators (SERMs) based on affective binding values and interactions, Bazedoxifene is predicted to compete along with selected benzamide derivatives between endogenous hormones and RE-α through reversible competitive inhibition and making it more selective against estrogen alpha receptors.

LIST OF ABBREVIATIONS

ER: Estrogen Receptor
DBD: DNA Binding Domain
AF-1: Activation factor -1
AF-2: Activation factor -2
ERα: Estrogen Receptor alpha
ERβ: Estrogen Receptor beta
SERM: Selective Estrogen Receptor Modulator
LBD: Ligand Binding Domain
Baz: Bazedoxifene

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

HUMAN AND ANIMAL RIGHTS

No animals/ humans were used for studies

AVAILABILITY OF DATA AND MATERIALS

Not applicable

CONFLICT OF INTEREST

None

FUNDING STATEMENT

None

SUPPLEMENTARY MATERIAL

None

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