ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Molecular Modeling and Prediction of Pharmacokinetics of Some Substituted Benzothiazoles That Block Steroidal Sulphatase Inhibitors for the Treatment of Breast Cancer

Srimathi. R*, Priya. D, Anjana G.V, Sundar Rajan. T

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRMIST, Kattankulathur, Chennai, India.

***Corresponding Author: R. Srimathi**

Abstract

A series of fifteen substituted 2-phenyl 1, 3- benzothiazoles were designed to be an inhibitor of Steroidal Sulfatase, the enzyme responsible for conversion of Estrone sulfate (E1) to Estradiol (E2). Estradiol is the active steroid hormone found in circulation that leads to carcinogensis and results in breast cancer. The crystal structure of Human estrone sulfate 1P49 was explored for possible interactions to substituted benzthiazole.Herein, we report the interactions of amino acid residue Arg ^{98}, Thr ^{99}, His ^{290}, Lys ^{368}, Thr ^{291}as the best possible interactions that plays a role in inhibiting the binding of $Ca^{2+}$to the active site. Compound 7C and 8C are reported with better binding affinities.

Keywords: *Benzothiazole, Steroidal sulfatase, Ligand, Interactions.*

Introduction

Breast Cancer is the most common cause of deaths in women worldwide with an alarming increase in new cases approximately (2.1 million in 2018) as reported by WHO [1]. The current treatment therapies for breast cancer in practice include surgery, radiation therapy, chemotherapy and hormonal therapy [2]. It is reported that approximately 95% of breast cancers are hormone dependent [3].

The physiological changes that drive for breast cancer in post-menopausal women include an increase in active steroid hormone (Estradiol) in circulation which are intracrinally biosynthesized by P450 aromatase, Estrone sulfate (E1S)& 17-Hydroxy Steroid Dehydrogenase (17-HSD_1) [4]. An estrogen in the form of Estradiol (E2) is the most potent estrogen which plays an important role in carcinogensis by binding to ER receptors & activating the down-stream transcriptions [5].

There are two important pathways that lead to production of Estradiol (E2) in target tissues [1] i) aromatase pathway and ii) steroidal sulfatase pathway [6]. In Aromatase pathway, Androgens are transformed to estrogens, which increase the level of Estradiol in circulation. As in case of sulfatase pathway, conversions of Estrone (E1) occur by Estronesulphatase enzyme which increases the production of Estradiol (E2) & leads to carcinogenesis. 17 ß-Hydroxy Steroid Dehydrogenase (17-ß HSD) is integral to both the pathways and convert E1 to E2 [7].

Estradiol (E2), an active diol, results in carcinogenesis by either of the below mechanism i) Stimulation of cellular proliferation through receptor mediated hormonal activity ii) Exerting direct genotoxic effects by increasing mutation rates through CYP 450 mediated metabolic activation iii) Induction of aneuploidy.

Designing of an inhibitor to aromatase or steroidal sulfatase pathway will decrease the production of active steroid hormones thereby proving to be treatment for breast cancer. Our research interest focused more on steroidal surfatase inhibition as studies were

reported that, at molecular level 87% of breast cancer patients showed higher expression of STS mRNA levels in malignant tissues. Supportive evidence states that a 10 fold greater amount of estrogen orginates via sulfatase pathway rather than aromatase route [8]. Also 5- Androsteroidal a steroid that circulates in postmenapousal women binds to ER and stimulates the growth of ER^+ breast cancer cells.

So in our work, we have explored in designing of a novel inhibitor to STS which would pare way for more specific inhibition in malignant breast cells. Furthermore, exploration of STS inhibitor started in 1990's with the introduction of estorone-o-sulfamate [EMATE], a potent STS inhibitor. The ring modification resulted in first generation, second generation and third generation STS inhibitors and 2-Substituted benzothiazoles are group of scaffolds which are part of this discovery and have shown STS inhibition [9].

Based on the above literature studies, we have designed 15 derivatives of 2- phenyl 1,3benzathiazole nucleus with modifications of halogens and alkyl on its ring system. These derivatives were extensively studied for in-silico drug design to STS inhibition (1P49) to explore the possible anticancer mechanism of our compounds.

Materials and Method

Structural Optimization

The chemical structure of fifteen 2- Phenyl 1, 3- Benzothiazole derivatives were designed using ACD labs Chemsketch drawing software [6].Energy optimizations of the drawn structure were performed using Avagadro [10].

Protein Preparation

The X-ray Crystallographic structure of human placental Estronediol DHEA sulfatase was downloaded from RCSB protein data bank with PDB code: 1P49 [11] with resolution 2.6 Å was used for the molecular docking simulations. The protein was prepared using Schrodinger's Maestroapplication software [Academic Version] [12]. The catalytic amino acid FGly75 (formylglycine) was converted to gem-diol form using Protein Preparation wizard. The converted protein was imported to Autodock tools 1.5.6 [13, 14] and the protein prepared.

The polar hydrogen atoms were added and gasteigercharges were added to added atom. The Non-Polar hydrogen atoms were merged to the protein structure and the prepared protein was saved in PDBQT files format for docking studies in Autodock 1.1.2 software. The STS inhibitors presented in Table 1 were sketched using ACD labs chemsketch drawing software and then energy minimized using Avagadro software. The ligands were imported and polar hydrogen atoms were added and gasteiger charges were assigned. The ligands were saved in PDBQT file format for performing docking.

Docking Studies:

The docking of the optimized inhibitors into the prepared rigid structure of the human steroid surfatase protein was performed using the Autodock 1.1.2 software. A grid box size of 60Å x 60Å x 60Å centered on the ß carbon atom of the amino acid FGly75 was use. The centre of the box was set at ligand centre and grid energy calculations were carried out. The Autodock calculations were performed using default parameters and 10 docked conformations were generated for each compound. The energy calculations were performed emplying Lamarckian Genetic Algorithm.

ADME Prediction

The Pharmacokinetics of the ligands was studied using pKcsm online software to study absorption, distribution, metabolism and Excretion of the designed ligands. The filter of Lipinksis rule of five was set for the ligands. [Table

Results& Discussion

Crystal structure RCSB: PDB: IP49 was downloaded and protein prepared. It is Human estrone sulfate that consists of total 562 amino acids and composed of only one A chain. The active site of STS is located deep in a cavity in the gill of the mushroom containing catalytic site.It consists of a transmemberane domain consisting of two antiparallel hydrophobic α helices.

These hydrophobic helices, each about 40Å long are capable of traversing the membrane, in such a way that the active site of globular domain resets near to the membrane surface, thereby suggesting a role of lipid bilayer in catalysis. The ligands were prepared and docked into the active site of the receptor

using grid box dimension of 60*60*60 A° and x=72.342, y=-1.523 and z=28.246 dimensions and the interactions studied. All the docked ligands show an interaction at Arg^{98} that is located in the third loop between residues 94 and 100 that serves as the "left swing door" to and from the active site.

Arg^{98} and Thr^{99} act as gatekeepers to the tunnel. It contacts with 17ß- hydroxyl end of substrate and there plays a significant role in recognition with our interactions, we confirm that ligand traverses to the active site by its interaction with Arg^{98} and Thr^{99} and marks for substrate recognition [Fig 1]. In the active site the catalytic amino acid hydroxyl formyl glycine FG (FGS). An electro density the Centre of the catalytic site requires cation (Ca^{2+}) for ES activity.

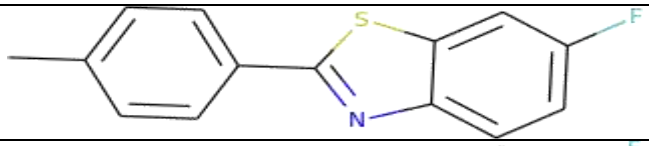
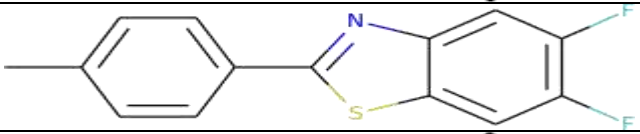
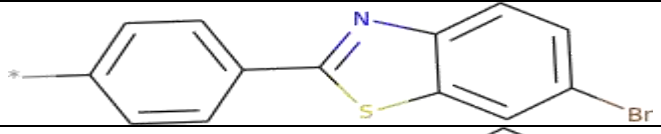
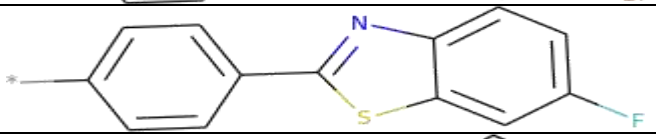
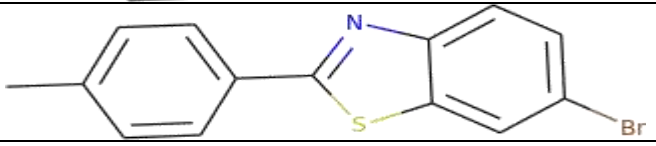
The imidazole ring of HIS 290 in compound 6C, 7C, 8C, 9C and 10C is located within a hydrogen bonding distance of (2.6 Å) away from the sulfate oxygen(Fig :2) .His 346 side chain is linked to Lys ^{368} and Thr^{291} side chain through hydrophilic interaction or by bridging of water molecule. A few additional solvent molecular like Glu349, Gly 100, His 485, val 177, Ser 97, Leu 74, ASN 447, Thr^{484}, (Fig: 3) which may be important for completion of hydrolysis are located inside the substrate-binding cavity E1 sulfate molecule is placed in the active site with its sulfate superimposed with FGs 75 which can be seen in Compound 1C, 2C and 6C.

The substrate binding end of the catalytic cavity has E1 sulfate molecule placed in active site with its sulfate superimposed with FGs 79. Residues Arg^{98}, Thr^{99}, Val^{101}, Leu^{103}, Val^{177}, Phe^{488}, Thr^{484}, Val^{486}, His^{485} surrounds the steroid backbone giving it hydrophobic contacts are recognized in the active site. Hydroxy formyl glyciene is covalently linked to sulfate moiety. In the resting state of the enzyme catalytic residue is present as sulfated hydroxyl formyls Lys 134, Ly 368, Arg 79, His 290 participate in catalysis in addition to their role in charfe neutralization inside the active site cavity.

Neucleophile attack on sulfur atom by one of the hydroxyl of hydroxylformyl glycine followed by activation by Ca^{2+}, whereas the other hydroxyl is deprotonated by His^{136}. This causes covalently linkage of sulfate moiety with formyl glyciene side chain and release of free E1. From the dock score, compound 6 C and compound 7C were found to have highest negative dock score of -8.21 and -7.78. It means that these formed most stable drug receptor complex amongst other compounds. All the docked compounds were analyzed for various types of interactions like hydrophobic bonding and compound were 6C and 7C for interaction exhibit hydrogen bonding with receptor.

The pharmacokinetics for the parameters was studied and results compared.

Table 1: Structures of the compounds with predicted IC50

Compound	Structure	Predicted IC50 (µM)
1C		18.45
2C		13.64
3C		16.32
4C		18.55
5C		2.41

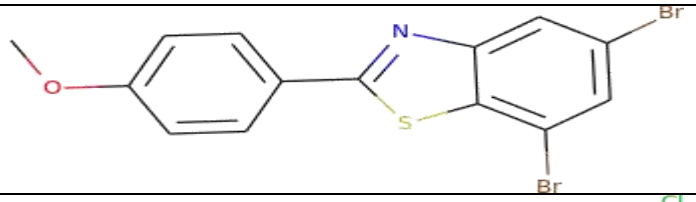
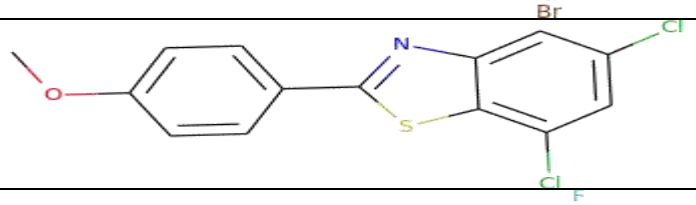
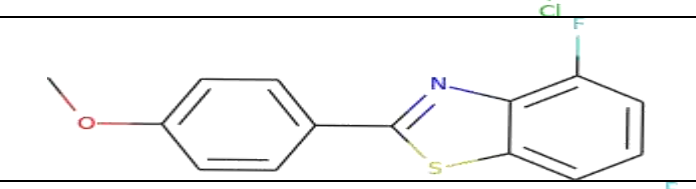
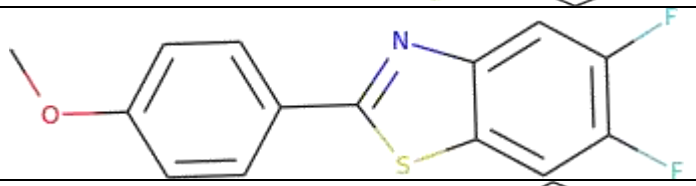
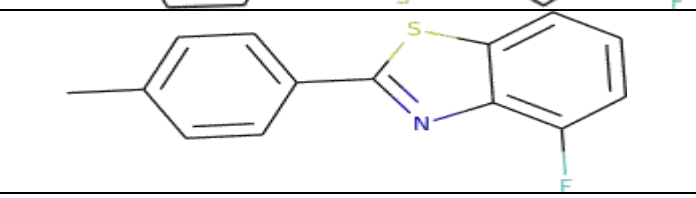
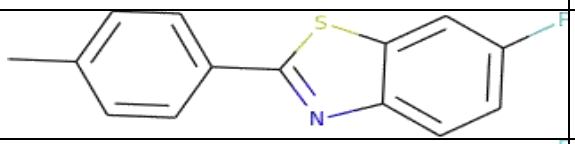
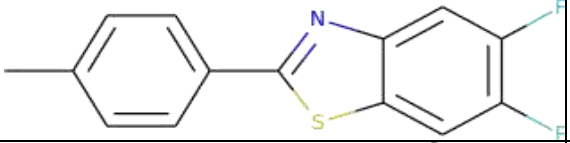
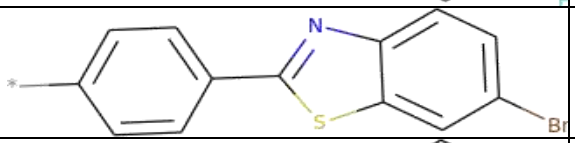
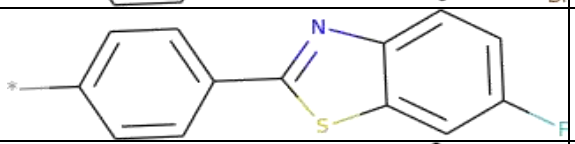
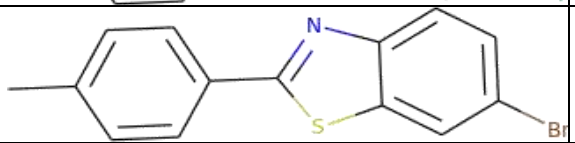
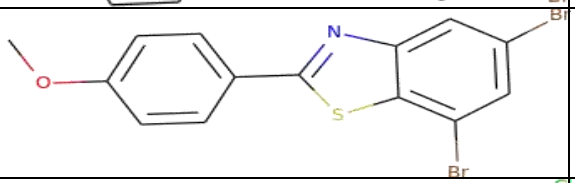
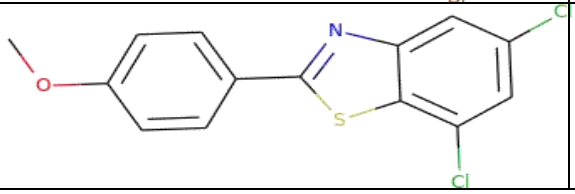
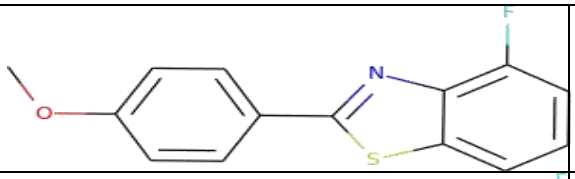
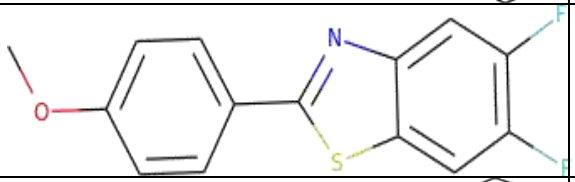
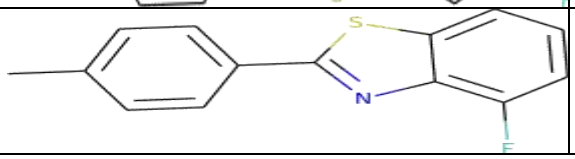
6C		966.31
7C		1.96
8C		9.93
9C		21.35
10C		7.69

Table 2: Lipinski's rule of Five:

Compound	Structure	Molecular Weight	Log P	Rotatable Bonds	Acceptors	Donors
1C		243.306	4.41082	1	2	0
2C		261.296	4.54992	1	2	0
3C		289.177	3.9004	1	2	0
4C		228.271	3.277	1	2	0
5C		304.212	5.03422	1	2	0
6C		399.107	5.4969	2	3	0
7C		310.205	5.2787	2	3	0

8C		259.305	4.111	2	3	0
9C		277.295	4.2501	2	3	0
10C		243.306	4.4108 2	1	2	0

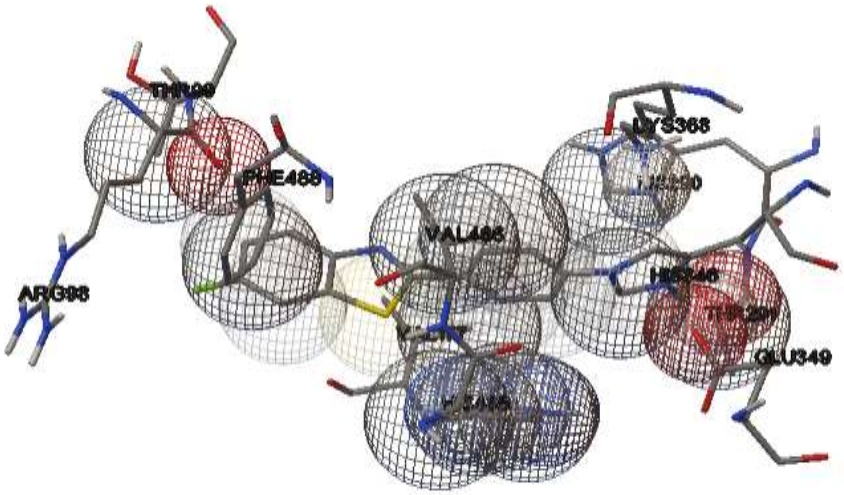


Fig 1: Arg [98] and Thr [99] Acting as Gate keepers to the tunnel

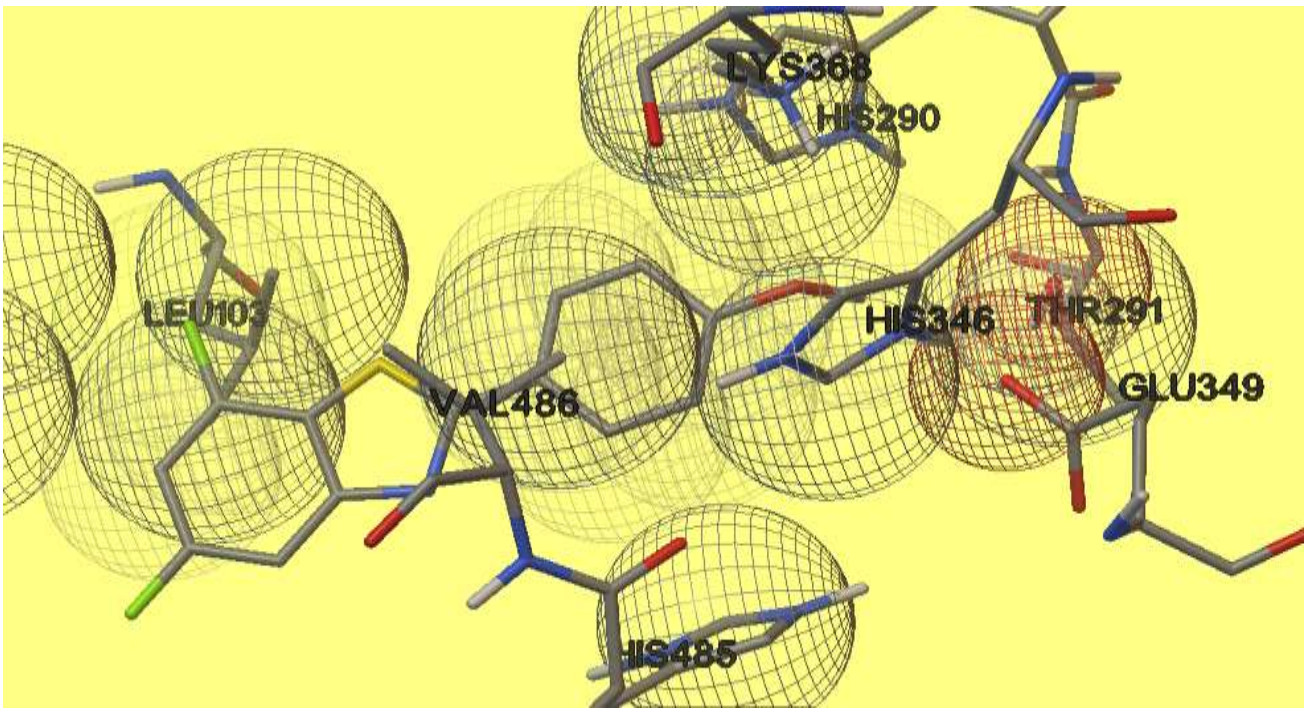


Fig 2: Compound 7 C showing His [290], Lys [368], Thr [291], His [346]interactions

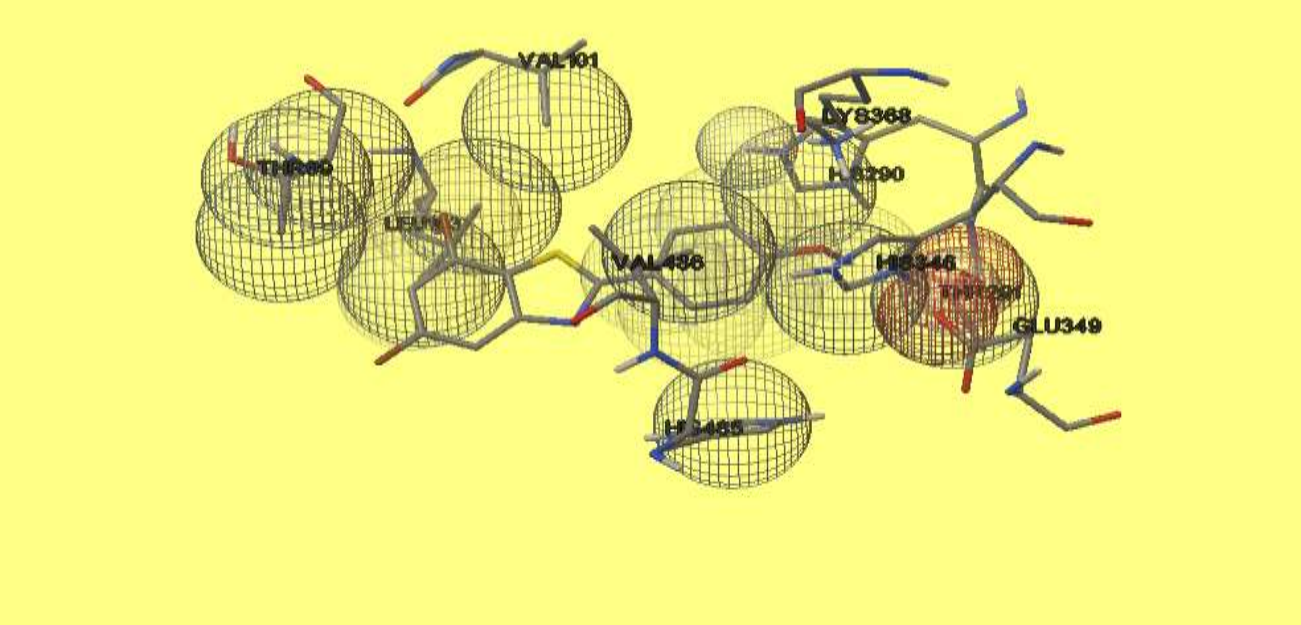


Fig 3: Compound 6C showing Glu ^{349} interaction

Conclusion

The substituted benzothizoles classified non-steroidal steroid sulfatase inhibitiors were selected for STS inhibition based on literature search. These ligands were sketched and docked into 1P49 the transmembrane Estrone sulfatase for inhibition. The docked conformations with binding energy from-6.37 to highest being -8.21 show better binding affinity for the ligands. These ligands also prove to be efficient by exposing the His 290 interactions at the active site which requires Ca^{2+} as is cation to form estrone sulfate. The designed molecules are predicted to bind by His 290 interactions and reduce the production of Estrone sulfate which inturn reduces the active steroid (Estradiol) hormone. The ligands are observed to follow Lipinskis rule of five proving it to be better in terms of Pharmacokinetics. Further exploration of these ligands by synthezing and evaluating against MCF-7 cell line would result in better molecules to mankind.

References

1. https://www.who.int/cancer/prevention/diagnosis-screening/breast cancer/en. Cancer. Accessed on:17.02.19

2. Ramanpreet Shah, Jatinder Singh, Dhandeep Singh, Amteshwar Singh Jaggi, Nirmal NM Singh (2016) Sulfatase inhibitors for recidivist breast cancer treatment: A chemical review. Eur. J. Med. Chem 114:170-190.https://doi:10.1016/j.ejmech.2016.02.054.

3. Parsaqualini J R (2004) The selective estrogen enzyme modulators in breast Cancer: A review.Biochim.Biophys.Acta.1654:123-143.

4. Billich A, Nussbaumer P, Lehr P (2000).Stimulation of MCF-7 breast cancer cell proliferation by estrone sulfate and dehydroepiandrosterone sulfate: inhibition by novel non-steroidal steroid sulfatase inhibitors. J. Steroid. Biochem. Mol. Biol., 73(5):225-235.https://doi:10.1016/S0960-0760 (00)00077-7.

5. Yager JD, Davidson NE (2006) Estrogen Carcinogenisis in breast cancer.N.Engl.J.Med.354:270-282.https://doi10-1056/NEJMra050776.

6. ACD/Structure Elucidator. Version 2018.1, Advanced Chemistry Development, Inc. Toronto, ON, Canada, www.acdlabs.com, 2019.

7. Xiaoyl Sang, Hui han, Donald Poirier, Sheng Xiang (2018) Steroidal sulfatase inhibition success and limitation in breast cancer clinical assays: an underlying mechanism. J. Steroid. Biochem. Mol. Biol. Accepted on:23-5-2018.https://doi10.1016/j.jsbmb.2018.05.009.

8. Purohit A, Woo LWL, Chander SK, NewMan SP, Ireson C, Ho Y, Grasso A, Lesse M P, Potter BV, Reed MJ (2003) Steroid sulphatase inhibitors for breast cancer therapy.J.Steroid.Biochem.Mol.Biol.:86:423-432.

9. Billich A, Schreiner EP, Wolff-Winiski. Benzoxa and Benzthiazoles. U.S Pat. Appl. US6716865 B1.

10. Avogadro: an open-source molecular builder and visualization tool.Version 1.XX.http://Avogadro.cc/

11. Franciso G, Hernandez-Guzman, Tadayoshi Higashiyama, Walter Pangborn, Yoshio Osawa, Debashis Ghosh (2003). Structure of Human Estrone Sulfatase Suggests Functional roles of membrane association. J. Bio. Chem., 278(25):22989-22997.https://doi 10.1074/jbc.M211497200.

12. Schrodinger Release 2019-1: Canvas. Schrodinger, LLc, New York, NY, 2019.

13. Morris G M, Huey R, Lindstorm W, Sanner M F, Belew R K, Goodsell D S, Olson A J (2009) Autodock4 and Autodock Tools 4: automated docking with selective receptor flexibility. J. Computational Chemistry, 16: 2785-91.

14. Russo J, Russo H (2006) The role of estrogen in the initiation of breast cancer. J. Steroid. Biochem. Mol. Biol., (1-5):89-96.

