Table **9.1.** Comparison chart of in-silico and in-vitro anticancer studies of compounds

S.No	Compound code	QSAR & Docking	ADMET studies	NCI activity One Dose assay (60 cell lines)	MTT assay (HeLa cell line)
1	Sch.1 (5)	Mild	Good	-	Mild
2	Sch.1 (6)	moderate	Good	-	Mild
3	Sch.1 (7)	Moderate	Exceptional	-	Moderate
4	Sch.1 (10)		Good	-	Good
5	Sch.1 (12)	Moderate	Good	-	Good
6	Sch.2 (15)	Moderate	Mild	Mild (UO-31 renal cancer cell line)	-
7	Sch.2 (17)	Moderate	Mild	Mild (HOP-92 Non-Small Cell Lung Cancer cell line)	-
8	Sch.2 (25)	Mild	Mild	Mild (HOP-92 Non-Small Cell Lung Cancer cell line)	-
9	Sch.2 (35)	Good	Good	Mild (UO-31 renal cancer cell line & UACC-62 melanoma cell line	-
10	Sch.3 (38)	Moderate	Good	Moderate (LOX IMVI melanoma cancer) & (CAKI-1 renal cancer cell lime)	Mild
11	Sch.3 (39)	Moderate	Mild	-	Mild
12	Sch.3 (40)	Moderate	Good	Good (HOP-92 Non-Small Cell Lung Cancer cell line) & moderate (UO-31 renal cancer cell line	Moderate
13	Sch.3 (41)	Moderate	Good	-	Moderate
14	Sch.3 (48)	Mild	Good	Mild (renal cancer)	-
15	Sch.3 (54)	Good	Mild	-	Good

S.No	Compound code	QSAR & Docking	ADMET studies	NCI activity One Dose assay (60 cell lines)	MTT assay (HeLa cell line)
16	Sch.3 (58)	Moderate	good	Exceptional (HL-60 leukemia cell line); Good K-562 leukemia cell line & (SR Leukemia cell line)	Mild
17	Sch.4 (60)	Moderate	Mild	-	Moderate
18.	Sch.4 (63)	Mild	Good	-	Moderate
19.	Sch.4 (64)	Moderate	Good	Good (UO-31 Renal cancer cell line)	-
20	Sch.4 (67)	Moderate	Good	-	Good
21	Sch.4 (69)	Good	Mild	Exceptional (CCRF-CEM Leukemia cell line); Moderate (MDA-MB-468 Breast cancer cell line)	Good

The parameters taken in QSAR/Docking, NCI anticancer screening (60 cell lines) and 96 well MTT assay (HeLa cell line) are namely mild, moderate, good, very good and exceptional. The QSAR/docking parameters were assigned based on predicted activity scores of QSAR, docking scores, H-bond interactions with MET 374 amino acid at the active site, Pi-Pi stacking with heme prosthetic group, H-bond interaction with other amino acids at the active site. Parameters for NCI anticancer activity on 60 cancer cell line and 96 well MTT assay against HeLa cell line were assigned based on growth inhibition percentage below 20 %, above 20 %, above 30 %, above 40 %. The lethal killing effect is considered as exceptional. Parameters of Mild, Good and exception were considered for ADMET studies based on passive positive gastrointestinal absorption, bioavailability radar and Toxicity class of the compounds data. The comparison chart shows the correlation of in-silico & ADMET studies with the in-vitro studies of the compounds on different cancer cell lines (Table 9.1).

In the present study, ligand based 3D QSAR pharmacophore modeling and docking studies of non-steroidal AIs was done for a series of novel Benzothiazole, Benzimidazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole, imidazo[2,1-B][1,3,4]thiadiazole derivatives. 3D Pharmacophore model ARR.1 was chosen with PLS factor 4 as the best model because the Training set correlation with Partial Least Square factors gave the best overall significance of model and statistical significance and the test set prediction correlations. The aromatase inhibitor potency of proposed ligands was influenced by Hydrogen bond acceptor and electron withdrawing groups on aromatic rings which is represented in the vector score and on the 3D pharmacophore model (Figure 3.8). Benzothiazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole containing n-methyl benzimidazole derivatives exhibited good predicted activities. However imidazo[2,1-B][1,3,4]thiadiazole derivatives gave negative predicted activity values. Any fused heterocyclic ring on the hydrogen bond acceptor hindered the aromatase inhibitory potency. The most virtually potent compounds of proposed ligands are 41, 45 and 50 (Figure 3.12).

The molecular docking studies were carried out on 34 different molecules including Letrozole and Vorozole. The molecules with good docking score were chosen here. The activity of the molecules was analyzed based on the docking score and hydrogen bond interaction of the ligand with the receptor. It is interesting to know that S configuration of all ligands showed better docking scores compared to their R counterparts because of the S configuration ligands hetero atoms (thidiazole, benzothiazoles) are mostly exposed to important binding interactions, that is MET 374, ASP 309 and Heme prosthatic group in the active site. The docked ligands binding with different amino acids of receptor and differentially binding with heme prosthetic group of aromatase active site with H-bond interaction, pi-pi stacking and wanderwall interactions emphasizes not only selectivity but also the alternate binding interactions at the active site of aromatase. These alternate binding interactions may help overcome mutated amino acids irresponsiveness due to chronic therapy. These novel chemo-types could be useful in developing rationale non-steroidal molecules for the aromatase inhibitory activity.

The docking studies on aromatase enzyme (PDB: 3S7S) also have shown reversible interactions at the active site with good docking scores comparable to Letrozole and Exemestane. Most of the derivatives were found to have H-bond interactions with L-Aspartic

acid 309, Methionine 374, L-Alanine 306. The Pi-Pi interaction of thiadiazole ring with heme prosthetic group of aromatase, H-bond interactions with methionine 374 and pi-pi interactions with heme, phenyl ring of tryptophan and phenylalanine amino acids at the active site are essential for effective biological activity which was once again reinstated. The presence of p-substituted phenyl is essential to access in to aromatase active site through ALA 306 and ASP 309. The para-substitution with high electronegative atom like Oxygen, Cyano functional groups enables H-bond interaction with MET 374 at the active site which is crucial for selective aromatase inhibition. Further selective studies in understanding the mechanism of action for the anticancer activity are needed.

2. Major Contributions

Our strategy is directed toward designing a variety of ligands with diverse chemical properties hypothesizing that by adding alternative binding group such as 2-amino thiadiazole, benzothiazole, imidazo[2,1-b][1,3,4]thiadiazole, p-substituted phenyl groups, substitutions on methylene bridge, to create molecular hybrids that have potential anticancer activity. The identified Pharmacophore groups involes in allosteric binding at the active site, enhance bioavailability of the drug and impart effective anticancer potency. In this way, such substitution pattern could target different regions of the aromatase active site to create differentially selective molecules. For enhancing lipophilicity we intentionally introduced mesoionic heterocyclic group like thiadiazole, lipophilic groups like N-methylbenzoimidazole, benothiazole, imidazothiazole fused hybrids. The design of our ligands was done based on quantitative structure activity relationship (QSAR) and molecular docking studies of non-steroidal aromatase inhibitors (letrozole analogues). The dramatic changes made on letrozole analogues with various Pharmacophore groups have shown appreciable predictaed activity similar to Letrozole. 2-amino-5-substituted 1,3,4-thiadiazole, p-substitued phenyl groups in a fashion similar Letrozole binds to the heme prosthetic group and metionine 374 amino acid in the active site of aromatase. The bulkier extensions on 2-amino group on thiadiazole ring with benzothiazole enhanced the predicted activity, where as imidazothiadiazole extension on the same decreased the predicted activity. This is due to the restriction of free rotation of the fused heterocyclic ring at the active site especially at the hydrophobic region. Similarly, based on the 3D Pharmacophore based QSAR and molecular docking results, mono halogen substituted

benzothiazole derivatives gave better activity results when compared with dihalogen bearing benzothiazole derivatives because of the stearic hinderence at the entrance of the active site and presence of polar amino acids at the neck region of the chanel entrance to active site. The docking study of designed compounds supports the QSAR postulation that the active compounds were acting on the same enzyme target where aromatase inhibitor Letrozole acts confirming the molecular design of the proposed class of anti-tumor agents.

During the synthesis of 2-amino thiadiazole derivatives from respective p/m-substituted phenyl acetic acids, it was found that electron withdrawing bearing phenylacetic acid derivatives gave moderate to better yields of 2-aminothiadiazoles in shorter duration of reaction time when compared to electron donating group bearing phenyl acetic acid derivatives (Scheme-1). On the other hand, if there is an N-N type molecule like thiosemicarbazide acting as the nucleophile, the factor that determines which nitrogen attacks seems to be the nucleophilicity, rather than steric effects. Electron withdrawing substitution bearing phenylacetic acids enhanced the rate of reaction. This information is valuable if a range of substituted phenyl acetic acid derivatives are used in the in formation of thiadiazoles and may go towards explaining differing yields of final product. Thus introduction of 2-aminothiadiazole ring to the molecule was crucial as there was potential to introduce diversity into the molecule. The simple attachment of benzothiazole to 2-amino group of thiadiazole adds as potential Pharmacophore with delicate free bond rotation between the two heterocyclic rings. The freedom of rotation between these heterocyclic rings is one of the critical factor in enhancing the effective QSAR predicted activity as well as good docking score.

Similarly during the synthesis and characterization of halogen substituted benzothiazole derivatives by bromine in acetic acid catalyzed annulations from various thiourea derivatives, we have found moderate yields with dihalogen substituted substrates when compared with non-substituted and mono halogen substrates. The order of reactivity was seen to follow the trend of the electronic contribution of the substituents through either induction, resonance or a combination of the two.

This 2-amino group extension of the thiadiazole also made it possible to synthesize imidazo[2,1-b][1,3,4]thiadiazoles with simple reaction conditions and reflux with phenacyl

bromide. However the reaction time for the synthesis of most of the imidazo[2,1-b][1,3,4]thiadiazoles derivatives is long.

This 2-amino group on thiadiazole also acts as linker to conjugate with bifunctional chelating agents like p-NCS-benzyl-DOTA which acts as chelating agent as well as linker at room temperature in the presence of simple phosphate buffer. The 2-aminothiadiazole also extends its primary amine to covalently bond with lanthanide like Lu.

3. Scope of further work

The present research proposal aims at the design and synthesis of new series of heterocyclic rings like benzothiazole, benzimidazole 1,3,4-thiadiazole, imidazo[2,1-B][1,3,4]thiadiazole and thioureaderivatives as aromatase inhibitors in the treatment of cancer. The in-silico studies of ligand Based 3D QSAR Pharmacophore Modeling and Molecular docking studies were done to strengthen the rationale of the study. Finding of the in-silico studies have given some insights of selectivity of molecule and possible alternate binding interaction with important amino acids at the active site of aromatase. Based on the literature review different synthetic routes were adopted to synthesize aforementioned heterocyclic compounds and their derivatives. A new series of nitrogen-containing heterocyclic compounds viz. 5-(substituted)-1,3,4-thiadiazol-2-amine, 1-(5-substituted-1,3,4-thiadiazol-2-yl)-3-phenylthiourea, 1-(5-substituted)-1,3,4-thiadiazol-2-ylbenzo[d]thiazole-2-amine and 2,6-disubstituted imidazo[2,1-b][1,3,4]thiadiazole derivatives, were developed as potential anticancer agents. The synthesized compounds were tested for one dose assay (10 μM) at the NCI over 60 cell line panel. With thorough in-silico, ADMET studies and NCI preliminary screening, some of the selected potential molecules were tested for in-vitro 96 well MTT assay on HeLa cell line with Letrozole as positive control. The findings of this research have showed that compound Sch.1 (10), Sch.1 (12), Sch.3 (54), Sch.3 (58) and Sch.4 (69) with potent anti cancer activity against HeLa cell line equivalent to letrozole. Compound Sch.3 (58) and Sch.4 (69) have showed selective anti-leukemic activity. The key pharmacophore features of the synthesized derivatives that enhanced the acnticancer activity of the derivatives are presence of sp2 nitrogen in thiadiazole ring, Cyanophenyl or methoxy functional group substitution on thiadiazole ring of all the heterocyclic compounds. These preliminary biological screening

studies have given positive anticancer activity for these new classes of derivatives. Additional research studies like the mechanism of action of the anticancer activity of this new class of compounds are considered necessary. In future, green chemistry approach for thiadiazole synthesis by using neat conditions at high temperature (melting temperature of acid derivatives) through conventionl as well as microwave assisted pathway can be adopted. This way hazardous $POCl_3$ can be avoided. In the synthesis of halogen substituted benzothiazoles, transition metal catalyst which involves annulative C-S bond formation can be developed to avoid hazardous bromine and acetic acid (bronchial irritants). The reaction time to synthesize imidazo[2,1-b][1,3,4]thiadiazole is very long. The reactions can be developed in suchway that the reaction time is reduced to below 4 hours by the use of heterogenous catalysts like nano-ferrate as green chemistry synthetic pathway. All the reaction process can be improved by developing suitable green chemistry synthetic pathway. These groundwork studies illuminate a future pathway for research of this class of compounds enabling the discovery of potent antitumor agent.