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Research Article

Insilico of Novel 2-Mercaptobenzothiazole Derivatives

Synthesis, Characterization and Evaluation of Some Novel 2-Mercaptobenzothiazole Derivatives: In Silico and Experimental Approach

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Abstract: Recently, QSAR technique and molecular docking have become widespread in designing new series of compounds, depending upon the different models developed, before actually synthesizing and testing them. Due to the diverse activity profile of substituted Benzothiazole, it has become a pharmacophore of interest. The main motive of the study was to design some novel 2- Mercaptobenzothiazole molecules with potential anti-inflammatory effect. Thus, in order to get a better insight into the structural features of 2- Mercaptobenzothiazole, a series of analogues were taken from the literature and 2D, 3D QSAR models were developed and molecular docking and ADME studies were also performed. For 2D QSAR study, stepwise forwarding variable selection method, coupled with Partial Least Square (SWF-PLS) regression-based method, was used to generate the best 2D model giving r^2= 0.9425, q^2=0.9018, *F-value*=90.0118, *pred_*r^2=0.8976. In contrast, the forward stepwise variable selection *k*-nearest neighbour molecular field analysis approach was used to generate best 3D QSAR model (q^2=0.8171 and *pred_*r^2=0.7006). Docking studies were performed on 29 derivatives of 2-Mercaptobenzothiazoles. The ligands were prepared and docked against protein *S. aureus* TYrRS with a resolution of 3.2Å. The three derivatives K6, K18 and K20 were found to have the best docking scores (-48.44, -47.46, -48.52). Further, synthesis of these three derivatives was carried out. Spectral characterization was carried out by IR, ^{1}H NMR and ^{13}C NMR and all the structures were found to be in agreement with the data obtained. *In vitro a*nti-inflammatory evaluation of these compounds was carried out by following the protein denaturation method and HRBC membrane stabilization method. Compound K20 displayed potential anti-inflammatory activity, which may be attributed to the electronegative group on the phenyl ring substituted on the pyrazole part of the pharmacophore.

Keywords: 2D; 3D QSAR Studies; Docking; HRBC: Membrane Stabilization Method; Protein Denaturation Method; Anti-Inflammatory Activity and 2-Mercaptobenzthiazoles

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1. INTRODUCTION

Due to the wide distribution of heterocyclic compounds in nature, they possess a wide variety of physiological activities. Alkaloids, antibiotics, chlorophyll, amino acids, dyes, drugs enzyme and genetic material all contain heterocyclic rings.[1] Benzothiazole is an aromatic heterocyclic compound with a benzene ring fused with thiazole ring, containing two heteroatoms, nitrogen and sulphur.[2, 3] It is a weak base and widely found in drug discovery applications and medicinal chemistry. Because of its various biological activities, benzothiazole ring have been attracting the attention of many scientists these days.[4] It acts as an essential part of several terrestrial and marine natural compounds and possesses various biological activities such as antiglycation, antioxidant, antileishmanial, anticonvulsant, neuroprotective agents, β -glucuronidase inhibitor, anti-allergic agents, antimicrobial, anti-inflammatory, antiparkinsonian, antitumor, antiviral, antiglutamate, anticandious, antimycobacterial properties[1, 5, 6, 7]. The substitution at the second position in the benzothiazole ring results in the change of its biological activities and therapeutic applications.[8] Substituted benzothiazoles attract attention because their derivatives were used as imaging agents for β -amyloid plaques, LTD-4 receptor antagonist and inhibitors of stearoyl-coenzyme A δ -9 desaturase, photosensitizers, orexin receptor antagonist.[9] They also act as phosphodiesterase inhibitors and serine hydrolases inhibitor, anticancer, anti-inflammatory, anti-allergic, antibacterial, antifungal, antiparasitic, and antioxidant[10]. 2-mercaptobenzothiazole is also one of the substituted benzothiazole derivatives with wide variety of biological activities.[11, 12] Various derivatives of MBT such as S-acethydrazide hydrazone and S-acyl derivatives were found to have antimicrobial properties[13]. Earlier, MBT was found to be exploited for its industrial applications.[14] But nowadays different derivatives are reported in the literature possessing different activities viz., antimycobacterial,[15, 16] antivirals,[17] anti-inflammatories,[18] anthelmintic,[19] antiulcer,[20] activities. Apart from these they were also found to possess mechanism based potential enzyme inhibitory activities *viz.,* carbonic anhydrase inhibitory activity,[21] acyl coenzyme A cholesterol acyltransferase (ACAT) inhibitory activity,[22] selective monoamine oxidase (MAO) inhibitory activity,[23] cathepsin D inhibitory activity,[24] COX-2 inhibitory activity[25]. Synthesizing a series of compounds and predicting the inhibitory potentials is costly and time-consuming. So before synthesizing, developing a prediction method for assessing biological activities is a need of today's research. By generating QSAR model, the biological and other activities can be correlated with the chemical structure, and it becomes easy to design a new series depending upon the predictions before synthesizing and testing them.[26]Thus, in shortening the drug discovery process, computational techniques have a great effect. Multiple linear regression (MLR), artificial neural networks (ANN), principal component analysis (PCA), and three-dimensional based methods like comparative molecular field analysis (CoMFA), and hypothetical active site lattice (HASL) are the different methods used to generate 2D and 3D QSAR models.[27] The quantitative structure-activity relationship (QSAR) of benzothiazole analogues exhibits significant and selective cytotoxicity on the tumorigenic cell line. Statistical methods, the (MM+) molecular-mechanics, (DFT) density functional theory, and the QSPR equations were used to study the toxicity of cell line [28]. Molecular docking is a tool by which the ligand is docked on the protein structure in the different binding orientations and by means docking scores the binding strength is predicted.[29] With the help of this tool, one can explore many libraries for finding therapeutically effective molecules. The study not only gives an idea about receptor affinity but also helps in the selection of newer molecules.[30] The main motive of the study was to design some novel 2-Mercaptobenzothiazole molecules with potent anti-inflammatory effects. Thus, to get a better insight into the structural features of 2- Mercaptobenzothiazole, a series of analogues were taken from the literature and 2D, 3D QSAR models were developed. Based upon the QSAR observations, 29 molecules were designed hypothetically and molecular docking was performed by using the TYrRS protein from *S.aureus*. The present study is actually an attempt for finding novel 2- Mercaptobenzothiazole analogues while keeping in view their therapeutic safety and efficacy.

2. MATERIALS AND METHODS

Initially, a dataset of 21 molecules of 2-mercaptobenzothiazole derivatives were taken from a study carried out by Hem Lata Kaur *et al*. Molecular Design Suite (Vlife MDS 4.6. software package, from Pune, India) on a HP Pentium IV 2.80 GHz Processor/ Microsoft Win XP Home Edition system was used for the all *in silico* studies (2D, 3D) QSAR. BIOPREDICTA tool of Vlife MDS software was used for the molecular docking study. All the compounds' structures were drawn with the help of Chem draw ultra-version 12.0 and MDL Molfile format was used to save them. For the optimization merck molecular force field method was used. By keeping the RMSG (root mean square gradient) value to 0.01 kcal/mol Å and the iteration limit to 10,000 the energy of the sketched structures was minimized. The conformer was selected for each compound and used for low energy further study. All the chemicals used for synthesis and biological evaluation purchased from Sigma-Aldrich, Hi-media and SRL. Open capillary method was followed for calculating the melting point and was found to be uncorrected. The TLC was done on precoated silica plates and the reaction progress was observed. All the reagents used were of analytical grade. The chemical, 2-MBT, was used after the recrystallization. Infrared spectra were performed by Shimadzu IR spectrophotometer by using KBr pellets. Bruker Avance 400 NMR spectrophotometer was used to record proton nuclear magnetic resonance spectra (^{1}HNMR) and the values were expressed as chemical shift (δ values, ppm) and tetramethylsilane (TMS) was used as internal standard. LC–MS were recorded on ABI Sciex Triple TOF/TOF 5600 System with LC-MALDI. Carlo Erba 1106 CHN analyser was used for elemental analysis.

2.1 QSAR and In-Silico Studies

2.1.1 2D QSAR Study

Quantitative structure-activity relationship (QSAR) is a computer based study by which models are developed by taking into account the values of different physicochemical variables and the biological activity of synthesized derivatives. The relationship developed will further help in designing of new chemical entities. An optimization of drug is carried out with the help of these models. Thus, by early *Insilico* evaluation of different properties of the designed analogues, the activity profile, selectivity towards the receptor protein and the toxicity profile of the designed molecules can be verified. The present study aims to perform 2D QSAR studies on a data set of 2-mercaptobenzothiazole analogues and find out the different structural requirements. The model deduced from these investigations provides an insight into the structural

requirements that could aid in designing new potentially active anti-inflammatory compounds. [30]

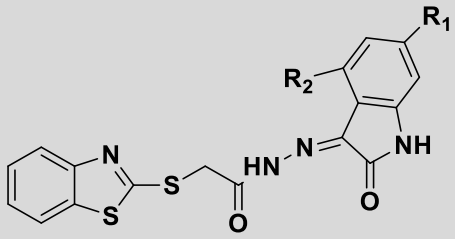
2.1.1.1 Dataset for 2D - QSAR study

Data of anti-inflammatory activity, in the form of structure and percentage inhibition of the compounds, was taken from the literature (Table 2.1).[31] The percentage inhibition was evaluated by means of *in vivo* carrageenan-induced rat paw oedema method. The percentage inhibition was converted into pIC_{50} by using formula: [32]

$$pIC_{50} = -\log C + \log it$$

Where C = molar concentration = [concentration (µg/mL) × 0.001/ molecular weight]
log it =log [inhibition/100 -%inhibition]

Table 2.1: The selected compounds for 2D and 3D QSAR studies



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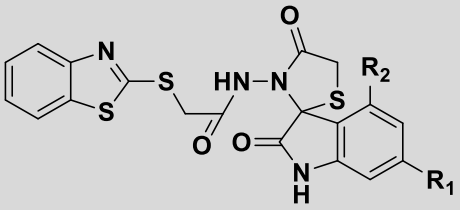

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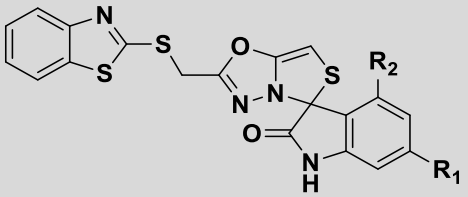


15-21

S.No.	R_1	R_2	pIC_{50}
1	$-OCH_3$	H	4.934838
2	CH_3	H	5.01395
3	H	CH_3	4.944314
4	Cl	H	5.156626
5	H	Cl	5.09933
6	Br	H	5.110021
7	H	Br	5.058868
8	H	$-OCH_3$	5.168612
9	CH_3	H	5.225346
10	H	CH_3	5.351463
11	Cl	H	5.414099
12	H	Cl	5.295501
13	Br	H	5.434867
14	H	Br	5.287739
15	$-OCH_3$	H	5.452766
16	CH_3	H	5.439979
17	H	CH_3	5.399415
18	Cl	H	5.614083
19	H	Cl	5.523906
20	Br	H	5.510816
21	H	Br	5.491511

The random selection method (RS) method was used to divide a set of total twenty-one analogues of benzothiazole before the QSPR model development. A training set comprising 14 molecules, a test set of 4 molecules for generating 2D QSAR models, and 10% molecules (3 molecules) to validate the quality of generated QSAR models were used. The test and training set selection is very important in the generation of QSAR models.

2.1.1.2 Descriptors Calculations

The minimum energy form is the most stable form for the effective binding of the drug to the receptor sites. In QSAR, the numerical depictions are used to represent molecules known as theoretical molecular descriptors, which further encodes the information regarding the molecular structure. These characteristics are very helpful for advanced research and theoretical molecular design. Various theoretical 2D individual descriptors such as vol., XlogP, and mol have been computed. wt; physiochemical parameters such as polar surface area, element count, hydrophobicity, estate numbers, estate contributions, dipole moment, logpA; XlogpA, hydrophobicity, and topological etc. To select the descriptors which are responsible for the anti-inflammatory activity of the derivatives a correlation matrix was applied in which each descriptor was taken as the independent variables and % paw oedema inhibition was taken as the dependent variable. By using QSAR tool module within Vlife Sciences Molecular Design Suite, 240 descriptors were computed. Using invariable column selection method, highly correlated descriptors or descriptors bearing similar values were first removed. Finally, 160 descriptors were used via stepwise forwarding variable selection (SWF) with the statistical methods including, Multiple linear regression (MLR), Partial least squares (PLS). And the 2D QSAR analysis resulted in optimum models with four significant descriptors. [33]

2.1.1.3 Statistical Analysis and Validation

Different variable selection methods such as Stepwise forwarding (SW), Genetic algorithm (GA) using MLR, PLS based regression/ algorithm developed a number of 2D QSAR models. For the generation of best QSAR models, those descriptors were selected that displayed the highest correlation with physiological activity. For the development of effective models, the arrangement of different parameters was carried out so that the regression equation consists of autonomous variables five times less than the total number of derivatives taken for the study. The program computes the best model on the basis of regression coefficient (r^2), which is an indicator of the variation in the values of experimental activity under consideration; internal cross validation regression coefficient (q^2), which is an indicator of the quality of fit; the external predictive power of the developed model is represented by the values of the external cross-validation regression coefficient (pred_r^2) and F-ratio indicated the variation between the calculated and observed activity and represented by Fischer's value F-test. The tabulated value of F-test showed 99.99% of degree of statistical importance when compared with counted value of F-test. Whereas the low values of standard error of pred_r^2, q^2, and r^2 represented how well the model is fit. The reproducibility of the QSPR models were represented by the internal validation (for pred_r^2) and cross validation (q^2) values. The fitness plot tells the statistical importance of the 2D QSAR model. A plot between the observed and predicted activity of training set and test set molecules is said to be the fitness plot. It not only gives the predicted activity of the external set but also tells how well the model was trained. The percentage contribution of the descriptors was used to derive the models, and the contribution chart of the model represented the descriptor contribution for building an effective QSAR model. [34]

2.1.2 3D QSAR Study

2.1.2.1 Dataset of 3D QSAR

For the development of 3D QSAR models, the same steps were followed for the 2D QSAR model development. The set of 21molecules was selected from the literature and was set according to the following ratio i.e. training: test: internal validation set: 14:4:3. Multiple conformations of each compound were generated by using Monte Carlo conformation search method. Conformations of the structure of the compound were searched by random selection method, and metropolis condition was used for the removal or acceptance of generated conformers. 4914 descriptors were produced and all descriptors having same values or zero values were eliminated before the model generation and resultant 4863 descriptors were used for carrying out the further steps. The descriptors were defined by calculating the molecular properties at the intersection point of a 3D frame in 3D-QSAR technique. In the ligand based 3D QSAR modelling method, molecular alignment is a prerequisite step to find productive results. Generally, there should be geometric alignment between the bioactive conformations and modelled structures of the selected molecules. [33, 34]

2.1.2.2 Alignment Procedure

The molecular alignment was performed to find productive results in the ligand-based 3D QSAR study. In Vlife MDS 4.6 software, template-based alignment method was used to align the data set of optimized and energy-minimized molecules. The alignment of a set of molecules is carried out by using a template structure. The conformational flexibility of the molecules is correlated with the moving of structure in 3D space which is a prerequisite in this method. A template is a common sub-structure of the series selected for the activity (Fig 2.1). The molecules were aligned on the reference structure, a biologically active stable conformation (Fig 2.2). All the structures found to be aligned with reference structure were selected for the study. The alignment procedure defined the most accepted pharmacophore for the series of ligands.

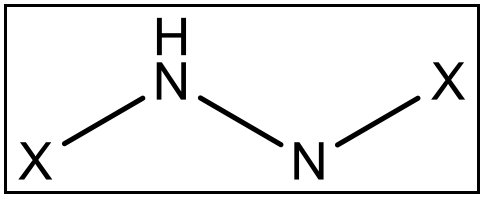


Fig 2.1: Template structure

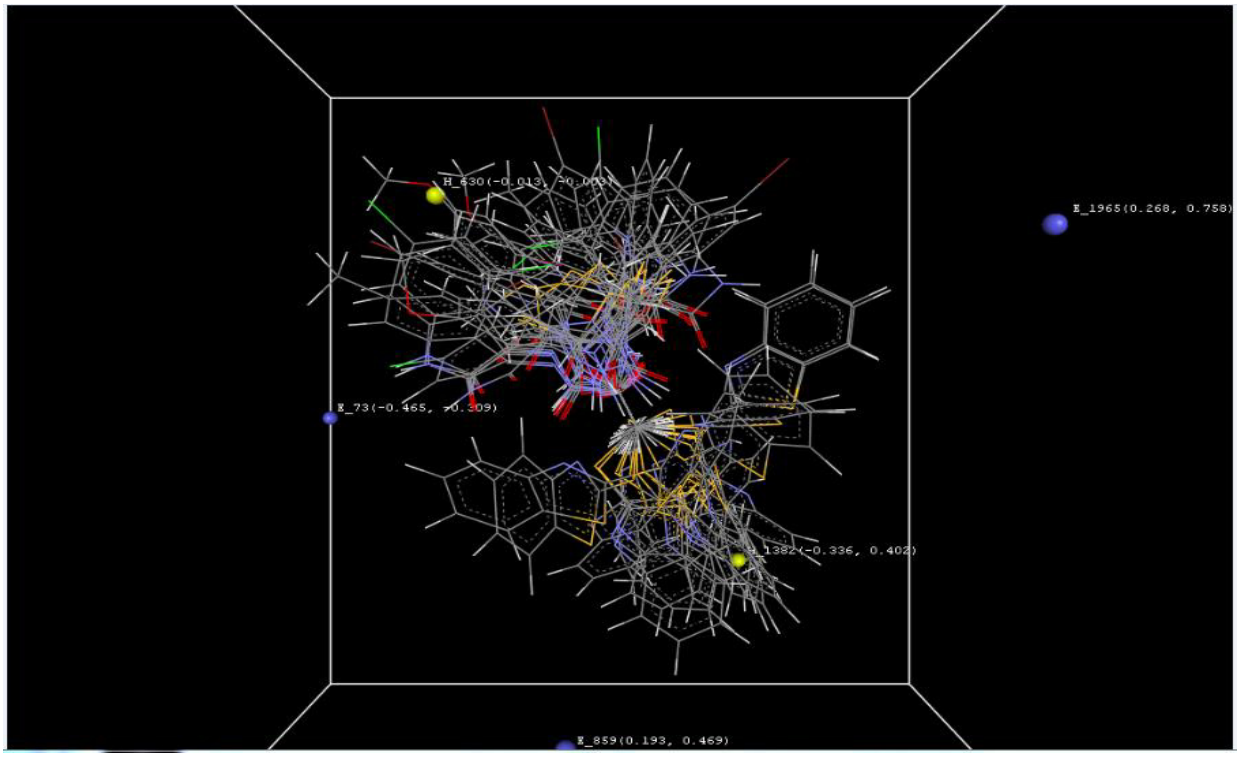


Fig 2.2: 3D view of aligned 2-mercaptobenzothiazole derivatives on template

2.1.2.3 Descriptors Calculations

The V life MDS 4.6 software was used to count physicochemical parameters of the aligned conformation after the optimization and energy minimization of the set of molecules. The probe, grid size, and grid interval were chosen for the generation of the descriptors. The distance-dependent dielectric constant was assigned a value of 1.0 which further lead to the calculation of different electrostatic, steric and hydrophobic descriptors for all the compounds in separate columns. In 3D-QSAR all invariable columns were removed because they were not playing any role in the generation of 3D-QSAR models.

2.1.2.4 k Nearest Neighbour (kNN) method

3D QSAR studies was performed by kNN method in which Forward Stepwise Variable Selection was used as variable selection method. The kNN method is based on the distance learning approach. In this method, a member which is unknown is selected among the majority of k nearest neighbours in the training set. The standard kNN-MFA method comprises the following steps.[35]

a. The distances between the training set's objects and the unknown (u) object were calculated.
b. Number of nearest neighbours (k) were selected, depending on the calculated distances.
c. By following leave one out (LOO) cross validation method, k value (an optimal value) was selected and the unknown object (u) was categorised among the group to which almost all the k objects belong.

Using the Stepwise Variable Selection method, the variables and optimal k values were selected. This variable selection method is used along with kNN method to standardize

(i) The number of neighbours (k) nearest to an unknown object and
(ii) The variables selected from the original pool.

In the initial stage, for developing a trial method a single independent variable was used, step by step the other independent variable was added, one at a time and model fitness was observed at every step until there were no more significant variables left to generate a best 3D-QSPR model—descriptors generated over grid, after generating the test and training sets by applied kNN method. Using a methyl probe of charge +1, the different energies (steric, hydrophobic and electrostatic) were computed at lattice points of the grid.

2.1.2.5 Cross Validation

The internal validation was performed by leave one out (LOO) method. In this method, a molecule from the training set was removed and to predict the activity by taking into account the average of the activities of the k most similar molecules, equation (1) was used. [35]

$$\hat{y}_i = \sum w_i \, y_i \qquad (1)$$

The elimination step was repeated until each molecule has been removed, predicting its activity. Further equation (2) was used to calculate r^2 and q^2 where y_i and $\hat{y}_i$ refers to the actual, and the predicted activities of the *i*th molecule and y_{mean} is the mean of observed activity of all the molecules in training set.

$$q^2 = 1 - \frac{\Sigma(y_i - \hat{y}_i)^2}{\Sigma(y_i - y_{mean})^2} \qquad (2)$$

The value 0.81 of q^2 indicated the internal stability of developed model which represented the effectiveness of kNN- MLR method and 2 was found to be optimum value of *k*.

2.1.2.6 External Validation

For carrying out the external validation, the equation (3) was followed:

$$\text{pred\_}r^2 = 1 - \frac{\Sigma(y_i - \hat{y}_i)^2}{\Sigma(y_i - y_{mean})^2} \quad (3)$$

wherein, y_i and $\hat{y}_i$ represents the actual and the predicted activities of the i th molecule in the test set and y_{mean} represents the mean of observed activity of all the molecules in training set. [35]

2.2 Molecular Docking

2.2.1 Methodology

It is a tool for finding the favorable binding position of the chemical molecules with the macromolecules. By analyzing the docking scores, one can predict how strong is the binding affinity or connection between the ligand and the protein molecule.[29] The important points to be taken care of while accessing the quality of docking technique:

(a) Observe true interaction mode of ligands to the target protein (docking accuracy)

(b) measure the relative changes in the observation of the real binding ligand utilizing a docking strategy against random screening (screening advancement). A series of 2-mercaptobenzothiazole (2-MBT) having twenty-nine derivatives have designed hypothetically based on QSAR observations. For assessing the different binding orientations, the 2-MBT derivatives and diclofenac sodium docked against protein from *S. aureus* TYrRS, and the system's energy was kept minimum. These designed molecules were sorted out for synthesis by using molecular docking. The molecular docking tools, Vlife MDS 4.6, software was used to observe the molecules' interaction mode into the active site of the protein from *S. aureus* TYrRS. (PDB ID:1JIJ)

2.2.2 Protein Preparation

The 3D crystal structure anti-inflammatory protein for *Homo sapiens* was not available, so docking was performed by using anti-inflammatory protein from *S. aureus* TYrRS (Fig 2.3), resembling the same as *Homo sapiens*, with a resolution of 3.2 Å which was obtained from protein data bank (PDB ID: 1JIJ) and saved in pdb format (www.rcsb.org). Molecular docking was performed after protein preparation and rectification followed by eliminating the water molecules, erasing the co-factors and ligands present in crystal structure, and optimizing of hydrogen bonding. The protein was made free from the external ligand and was saved in pdb format.

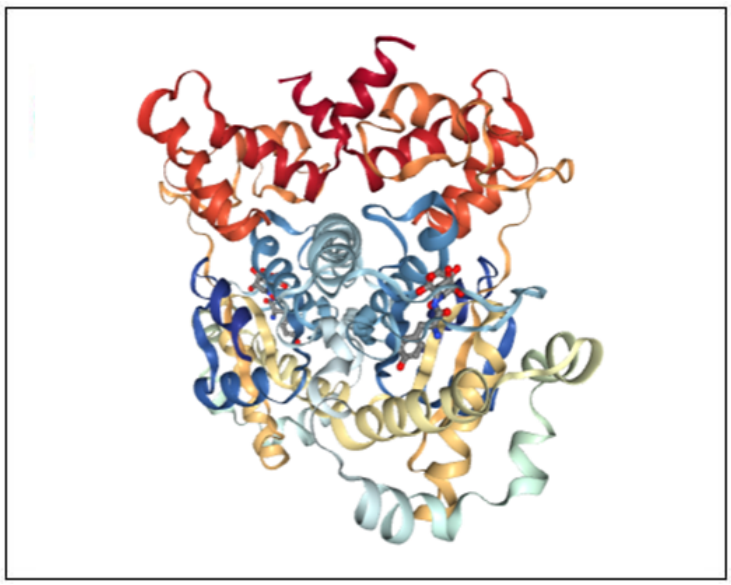


Fig 2.3. Structure of (S. aureus TYrRS); containing required protein chains and hydrogen bonds

2.2.3 Ligand Preparation

The key factor is the ligand's chemical structure for the docking to be effective. The 2D structures of the ligands were drawn with the help of Chem Draw Ultra-12.0, which were converted to 3D forms. While doing the refinement, by using the (MMFF) Merck Molecular Force Field technique and an analytical gradient, the energy of the co-crystallized complexes was stabilised and for further studies the lowest energy conformations were chosen.

2.2.4 Ligand Docking

An interest in theoretical *in silico* methods has emerged due to the evaluation of biological and chemical informatics in conjunction with the development of specialized software and growing computer applications. The ability to bridge the chemical and biological spaces is essential for discovering and developing new drugs. A complete and integrated software suite for computer assisted chemical and drug development is Vlife MDS 4.6. Docking analyses on a group of molecules will not only help discover hits or leads but also provide an insight

into crucial structural characteristics for lead optimization. Using an advanced docking software, lower energy conformers of 29 molecules of 2-mercaptobenzothiazole derivatives were docked on the *S. aureus* TYrRS receptor. Docking experiments were carried out using the docking score (D.S.) and as a result the poses for the ligand (L.P.) were assigned as shown in (Table 2.2). Following a docking simulation, the best-docked conformer of the ligand was examined for different interactions in the binding sites, including van der Waal's interaction, aromatic interactions, hydrophobic bonding, and H-bonding. Analysis of simulated binding was performed on the docking of *S. aureus* TYrRS complexes of 2-mercaptobenzothiazole derivative (D-score). [29]

2.3 ADME Studies

As there are number of promising candidates with good docking score but are generally withdrawn during clinical trials, because of their inability to satisfy various pharmacokinetic parameters. In the preceding study, all the three candidates were gone through ADME studies using online https://preadmet.webservice.bmdrc.org/ server. This approach is anticipated to be useful in early lead identification in combination with drug target interaction (DTI).

2.4 Chemistry

***Synthesis of ethyl 2-(benzo[d]thiazol-2-ylthio acetate [I]*:** 2 gm of 2-mercaptobenzothiazole, 15 mL dry acetone and 2 gm of potassium carbonate were taken in a round bottom flask and mixed. Then 2 mL ethyl chloroacetate was added with continuous stirring into it and refluxed the reaction mixture for 8 h using oil bath at 48^{0}C. Further, it was poured on crushed ice. The reaction mixture was neutralized by adding a few drops of hydrochloric acid. The needle-shaped crystals were collected after the filtration of the reaction mixture. [36]

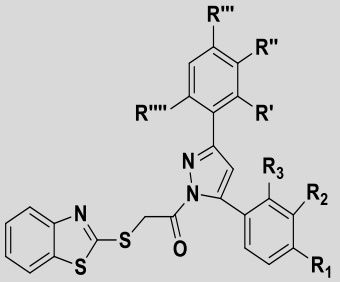
(I) Ethyl 2-(benzo[d]thiazol-2-ylthio) acetate IR (KBr, cm^{-1}): 3064.95 (C-H str., aromatic), 2977.64 (C-H str., aliphatic) 1737.19 (C=O str., ester), 1459.79 (C=C str., aromatic), 1368.20 (CH_3 bend), 1298.75 (C-O-C str.), 599.68 (C-S str.). H^{1}NMR: (DMSO) δ (ppm): 1.26 (3H, t, CH_3), 4.23 (2H, q, CH_2), 4.49 (2H, s, CH_2), 6.73-7.83 (4H, m, ArH). MS (m/z): 371 (M+), 373 (M + 2).

***Synthesis of 2-(benzo[d]thiazole-2ylthio) acetohydrazide [II]*:** Compound (I) (1 mol) and hydrazine hydrate (0.4 mol) and ethyl alcohol (30) mL were mixed in RBF and refluxed for 14 h in oil bath at 50-55 ^{0}C. The mixture was cooled, and the solvent was evaporated on a rotary evaporator. The final solid product was filtered, dried, and washed with water several times to obtain compound (III). [36]

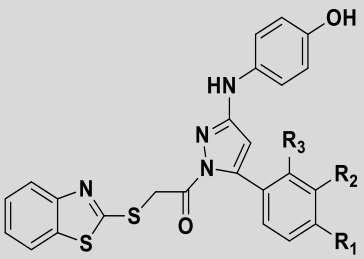
(II) 2-(benzo[d]thaizol-2-ylthio) acetohydrazide IR (KBr, cm^{-1}): 3285.52 (N-H stretching), 3199 (C-H str., aromatic), 2867.67 (C-H str.), 1646.73 (C=O amide), 1559.55 (C=C str., aromatic) 1452.54 (C=C bend, aromatic), 646.08 (C-S str.). H^{1}NMR (DMSO) δ (ppm): 2.16 (s, 2H, NH_2), 4.42 (2H, s, CH), 6.70-7.80 (4H, m, ArH), 8.91 (s, 1H, NH).

***Synthesis of Chalcones [compound III (A-C)]*:** The equimolar quantity of various acetophenones (0.02 mol) and different benzaldehyde (0.02 mol) were taken and dissolved in ethanol (10mL). 10% of aqueous NaOH solution (12 mL) was poured drop-wise into it with constant stirring at 20-25°C temperature and stirred the reaction mixture for 3-4 h. TLC checked the reaction progress. After completion of the reaction, 300 mL of water was poured in the reaction mixture. The reaction mixture was constantly stirred while addition of water. While using hydroxyl acetophenones, the reaction mixture was neutralized by 0.02N HCl, resulting in precipitating formation. The formed precipitates were filtered, washed with water, and then recrystallized with ethanol. [37]

Table 2.2. Structure of various compound with their Docking Score and ligand pose



K1-K26



K27-K29

S.NO.	R_1	R_2	R_3	R'	R''	R'''	R''''	Ligand Pose	Docking Score
K1	H	H	NO_2	H	H	H	H	5	-35.150
K2	H	F	H	H	H	H	H	1	-34.50
K3	H	F	H	H	H	I	H	3	-22.83
K4	H	F	H	OH	H	OH	H	3	-40.23
K5	NO_2	H	H	H	H	H	H	2	-45.00
K6	F	H	H	H	H	H	H	4	-48.44
K7	H	H	H	H	H	I	H	3	-34.57
K8	H	H	NO_2	H	H	I	H	2	-38.12
K9	NO_2	H	H	H	H	I	H	2	-35.66
K10	F	H	H	H	H	I	H	3	-37.88
K11	H	H	H	H	H	OH	OH	2	-19.67
K12	H	H	NO_2	H	H	OH	OH	1	-34.63
K13	H	NO_2	H	H	H	OH	OH	3	-37.63

K14	NO_2	H	H	H	H	OH	OH	3	-24.52
K15	F	H	H	H	H	OH	OH	5	-28.10
K16	H	H	OH	H	H	H	H	2	-36.78
K17	$-OCH_3$	OH	H	H	H	H	H	4	-39.98
K18	H	H	OH	OH	H	H	H	3	-47.46
K19	OCH_3	OH	H	OH	H	H	H	4	-37.54
K20	H	H	H	OH	H	H	H	5	-48.52
K21	H	H	H	H	H	OH	H	I	-21.70
K22	$-OCH_3$	OH	H	H	H	OH	$-OCH_3$	3	-20.91
K23	H	H	H	H	OH	$-OCH_3$	H	2	-38.10
K24	H	H	F	H	H	I	H	3	-35.54
K25	H	H	F	H	H	H	H	I	-38.79
K26	H	H	H	OH	H	OH	H	4	-32.85
K27	H	H	H	-	-	-	-	4	-34.70
K28	H	H	OH	-	-	-	-	2	-37.82
K29	$-OCH_3$	OH	H	-	-	-	-	I	-13.68

III (A) (E)-3-(4-fluorophenyl)-1-phenylprop-2-en-one IR (KBr, cm^{-1}): 3060.48 (C-H, str., aromatic), 2929.06 (C-H str., aliphatic), 1659.42 (C=O str., ketone), 1596.46 (C=C str., aliphatic), 1506.61 (C=C str., aromatic), 1214.77 (C-F str.), 979 (CH, oop bend, alkene). H^1NMR ($CDCl_3$) δ (ppm): 7.321 (m, 2H, ArH), 7.583(m, 2H, ArH), 7.682 (m, 1H, ArH), 7.779 (d, 1H, J=15.68, CH), 7.920 (d, 1H, J=15.68, CH), 8.001(m, 2H, ArH), 8.164 (m, 2H, ArH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 190.56, 163.01, 143.71, 135.05, 132.14, 131.06, 130.59, 130.50, 129.85, 128.67, 128.32, 121.12, 116.42 and 116.21. Anal. Calcd for $C_{15}H_{11}FO$ (226.25): C, 79.63; H, 4.90. Found: C, 79.60, H, 4.93.

III (B) (E)-1,3-bis(2-hydroxyphenyl) prop-2-en-1-one IR (KBr, cm^{-1}): 3429.14 (OH str.), 3200.00 (C-H str., aromatic), 3061.30 (C-H str., alkene), 1679.19(C=O str., ketone), 1605.42 (C=C str., alkene), 829.64 (C-H oop, bend, alkene). H^1NMR ($CDCl_3$) δ (ppm): 6.783 (m, 4H, ArH), 7.141 (m, 2H, ArH), 7.756 (m, 2H, ArH), 7.401 (m, 1H, CH), 8.152 (d, 1H, CH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 190.13, 162.12, 158.50, 146.11, 136.80, 131.56, 130.12, 128.01, 122.90, 122.12, 121.56, 121.20, 116.21 and 114.92. Anal. Calcd for $C_{15}H_{12}O_3$ (240.25): C, 74.99; H, 5.03. Found: C, 74.94, H, 5.06.

III (C) (E)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one IR (KBr, cm^{-1}): 3431.11 (O-H str.), 3036.32 (C-H str., aromatic), 2898.49 (C-H str., aliphatic), 1690.54(C=O str., ketone), 1605.72 (C=C str., alkene), 785.82 (CH oop bend, alkene). H^1NMR ($CDCl_3$) δ (ppm): 7.081 (m, 2H, ArH), 7.226 (m, 3H, ArH), 7.433 (m, 3H, ArH), 7.632 (m, 2H, CH), 8.101 (m, 1H, ArH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 190.11, 162.35, 137.34, 131.83, 129.01, 128.95, 126.80, 122.95, 122.23, 121.90 and 116.92. Anal. Calcd for $C_{15}H_{12}O_2$ (224.25): C, 80.34; H, 5.39. Found: C, 80.36, H, 5.34.

Synthesis of K (6, 18, 20): In round bottom flask, the equal molar quantity of chalcone and compound (III) were taken and 40 mL of ethanol was added. Few drops of glacial acetic acid were added. Hydrochloric acid was used as catalyst in the reaction, the pH of the reaction mixture was maintained up to 5-6. The reaction mixture was refluxed for 19hr at 58-65 ^{0}C. TLC was carried out by using precoated silica plates and suitable solvent system to check the progress of a reaction. TLC was observed inside the UV-chamber and potassium permanganate stain. When reaction was completed, the mixture was poured onto crushed ice. The resultant product was kept overnight and then separated, filtered, washed with water and dried. [36]

(K6)2-(benzo[d]thiazol-2-ylthio)-1-(5-(4-fluorophenyl)-3-phenyl-1H-pyrazol-1-yl)ethanone IR (KBr, cm^{-1}): 3066.65 (CH- str., aromatic), 2935.86 (C-H str., aliphatic), 1735.07 (C=O str., amide) 1678.71 (C=N str.), 1600.21 (C=C str., aromatic), 1268.77 (C=N-N), 1216.93 (C-F str.), 690.11 (C-S str.).H^1NMR ($CDCl_3$) δ (ppm): 8.24 (s, 2H, ArH), 8.037-8.058 (m, 2H, ArH), 7.974 (d, 1H, J=7.6 Hz, ArH), 7.651-7.686 (m, 3H, ArH), 7.512-7.567 (m, 3H, ArH), 7.121-7.197 (m, 2H, ArH), 7.015 (d, 1H, J= 8.0, ArH), 6.891 (s, 1H, CH pyrazole), 4.101 (s, 2H, CH_2). ^{13}C-NMR ($CDCl_3$) δ (ppm): 190.56, 158.83, 150.85, 143.71, 130.05, 132.14, 131.06, 130.59, 130.50, 128.85, 128.67, 126.95, 122.0., 121.80, 121.12, 119.42, 117.47, 116.44, 116.23 and 35.42. Anal. Calcd for $C_{24}H_{16}FN_3OS_2$ (445.53): C, 64.70; H, 3.62; N, 9.43. Found: C, 64.67, H, 3.64, N, 9.41.

(K18)2-(benzo[d]thiazol-2-ylthio)-1-(3,5-bis(2-hydroxyphenyl)-1H-pyrazol-1-yl) ethanone IR (KBr, cm^{-1}): 3394.19 (O-H str.), 3200.68 (C-H str., aromatic), 3056.14 (C-H str., aliphatic), 1663.96 (C=O str., amide), 1609.61 (C=N str), 1571.54 (C=C str., aromatic), 1271.80 (=N-N str.), 756.53 (C-S str.). H^1NMR ($CDCl_3$) δ (ppm): 7.906-7.933 (m, 2H, ArH), 7.670-7.739 (m, 3H, ArH), 7.112-7.485 (m, 7H, ArH), 6.883-6.989 (m, 1H, CH, pyrazole), 5.08 (s, 2H, OH), 4.14 (d, 2H, J=14.0, CH_2). ($CDCl_3$) δ (ppm): 147.10, 139.42, 131.77, 128.93, 128.74, 126.26, 126.08, 125.84, 124.80, 121.85, 120.45, 117.54 and 35.26. Anal. Calcd for $C_{24}H_{17}N_3O_3S_2$ (459.54): C, 62.73; H, 3.73; N, 9.14. Found: C, 62.70, H, 3.74, N, 9.11.

(K20)2-(benzo[d]thiazol-2-ylthio)-1-(3-(2-hydroxyphenyl)-5-phenyl-1H-pyrazol-1-yl) ethanone IR (KBr, cm^{-1}): 3429.14 (O-H str.), 3178.96 (C-H str., aromatic), 3055.27 (C-H str., aliphatic), 1654.33 (C=O str., amide) 1620.33 (C=N str.), 1459.02 (C=C str., aromatic), 1200.65 (=N-N str.), 761.09 (C-S str.). H^1NMR ($CDCl_3$) δ (ppm): 8.236 (d, 1H, J= 8.4 Hz, ArH,), 7.939 (t, 1H, J= 8.4Hz, ArH), 7.828 (t, 2H, J=8.4 Hz, ArH), 7.510 (t, 1H, J=7.6 Hz, ArH), 7.398 (d, 2H, J= 8.0 Hz, ArH), 7.297 (t, 1H, J= 9.2, ArH), 7.176 (t, 1H, J= 8.0Hz, ArH), 6.987 (t, 2H, J=8.4 Hz, ArH), 6.849-6.888 (m, 1H, CH, pyrazole), 4.102 (d, 2H, J_{gem} =9.2 Hz, CH_2). ^{13}C-NMR ($CDCl_3$) δ (ppm): 131.85, 130.84, 126.62, 125.08, 121.51, 121.02, 119.73, 119.20, 117.19, 40.31, 39.90, 39.69 and 35.32. Anal. Calcd for $C_{24}H_{17}N_3O_2S_2$ (443.54): C, 64.99; H, 3.86; N, 9.47. Found: C, 64.95, H, 3.84, N, 9.42.

2.5 Anti-Inflammatory Evaluation

2.5.1 By Protein Denaturation Method

The mixture (5 mL) contains phosphate buffer saline (PBS, pH 6.4) 2.8 mL, egg albumin 0.2 mL, and 2 mL of varying concentrations of 62.5, 125, 250, 500, 1000 µg/mL. Diclofenac

sodium utilized as standard drug. Equal volume of DMSO (dimethyl sulphoxide) filled in as control. Incubated the mixtures for five minutes at (37 ^{0}C.) in BOD (Labline Technologies). In the wake of cooling, their absorbance was estimated at 660 nm (SHIMADZU, UV 1800) by taking DMSO as blank. The formula followed for the calculation of percentage inhibition given below.[38]

$$\% \text{ inhibition} = 100 \times (Vt/Vc - 1)$$

where, Vc = absorbance of control and Vt = absorbance of test

2.5.2 By HRBC (Human Red Blood Cell Membrane Stabilization) Method

The blood from the healthy human volunteer was used for the study and ensured no NSAID had been consumed for the past two weeks. The blood was mixed with equal volume of Alsever solution (sodium citrate 0.8%, dextrose 2%, NaCl 0.42% and citric acid 0.5%). The mixture was then centrifuged at 3,000 rpm. Isosaline (0.85%, pH 7.2) was used to wash the packed cells and a suspension of 10%v/v was made in isosaline. By using DMSO, different concentration was prepared (62.5, 125, 250, 500, 1000 µg/mL). Every concentration was mixed with 1 mL of phosphate buffer (0.15 M, pH 7.4), 2 mL hyposaline (0.36%) and 0.5 mL of HRBC suspension. Diclofenac was used as the standard drug. All the concentrations were incubated at 37°C for 30 min and again centrifuged for 20 min at 3,000 rpm. The haemoglobin content in the supernatant solution was assessed spectrophotometrically at 560 nm. The following formula calculated the percentage protection or HRBC membrane stabilization.[39]

$$\% \text{ protection} = 100 - (\text{O.D. of sample} / \text{O.D. of control}) \times 100$$

where O.D. = optical density.

3. RESULTS AND DISCUSSION

3.1 2D QSAR

The role of 2-mercaptobenzothiazole derivatives as anti-inflammatory agents was found to be very promising. Therefore, QSAR studies were carried out to explore the potency of the synthesized derivatives. The study was performed by using the QSAR module in Vlife MDS 4.6 software. The study develops various 2D QSAR models by following different statistically significant regression-based methods such as multiple regression method (MLR), and partial least square method (PLS) coupled with different variable selection methods. The study resulted in the development of the three best models. Using 2D QSAR studies, one can predict the activity of molecules. The performance of the QSAR studies depends upon the selection of logical descriptors. Moreover, the selection of these descriptors, which proved to be significant in developing the models with significant predictive power was carried out with help of PLS, MLR techniques. The best-fit model was found to have a minimum number of descriptors. The three models which gave the best results were SWF-MLR (Model 1), SWF-PLS (Model 2) and SWF-PLS (model 3). The values for r^2 and q^2 shown in Table 3.1 has justified the predictive ability of 2D QSAR study.

Model 1 (SWF-MLR)

% oedema inhibition **=** +5.0758(XlogP) +5.1624(SsCIE-index) ……..…Equation 1

N = 21, r^2= 0.9249, q^2= 0.8931, pred r^2= 0.8772, F test = 67.7708, r^2se = 0.4132, q^2se = 0.4226, pred r^2se = 0.4832

Model 2 (SWF-PLS)

% oedema inhibition **=** +5.7454(XlogP) + 9.4050(SsCIE-index)-2.1753(Xcomp

Dipole)-1.0871(Dipole moment) …… Equation 2

N = 21, r^2= 0.9544, q^2= 0.8319, pred r^2= 0.7090, F test = 115.134, r^2se = 0.4506, q^2se = 0.4361, pred r^2se = 0.5812

Model 3 (SWF-PLS)

% oedema inhibition **=** +5.3795(XlogP) +6.6604(SsCIE-index)-0.0990

(XKHydrophobic area) …… Equation 3.

N = 21, r^2= 0.9425, q^2= 0.9018, pred r^2= 0.8976, F test = 90.0118, r^2se = 0.4036, q^2se = 0.4125, pred r^2se = 0.4765

From the data, it has been obvious that out of the three best models, Model 3 was found to be the most statistically significant. The value of regression coefficient r^2 i.e. 0.9425 indicated 94.25% of the variability in the experimental activity. The q^2 (0.9018) cross-validation coefficient indicated the fitness of the model means the internal predictive ability of the

model is 90.18%. And the value of pred r^2 is 0.8976 which is the maximum of all the generated models, indicating the model's good external predictive ability. Whereas the low values of the standard error (r^2se = 0.4036, q^2se = 0.4125, pred r^2se = 0.4765) displayed how accurate the model is. The F-test with value of 90.0811 demonstrated that the model is 99.99 percent statistically significant. The Fig. 3.1 provided the contribution of various descriptors and the plot for fitness of the model. The fitness of the model is further supported by the overlapping of Radar plots shown in Fig. 3.2.

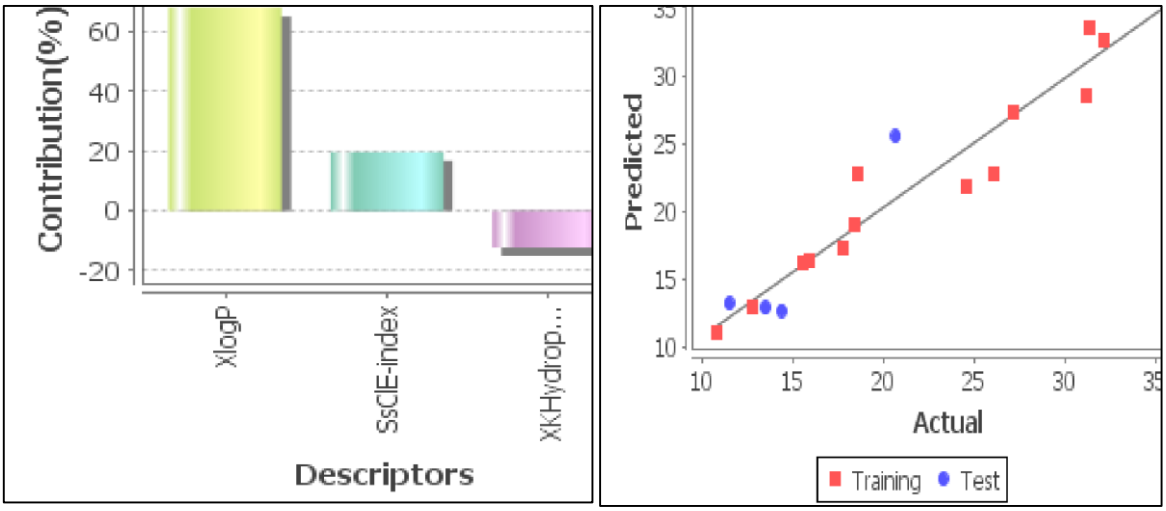
Table 3.1. The different models generated for better correlation by statistical validation

Models	SWF-MLR (Model 1)	SWF-PLS (Model 2)	SWF-PLS (Model 3)
F-value	67.7708	115.134	90.0118
Predicted r^2	0.8772	0.7090	0.8976
q^2	0.8931	0.8319	0.9018
r^2	0.9249	0.9544	0.9425

The above table signifies the development of 2D QSAR models by using stepwise forward selection method. The statistical methods used were MLR (multiple linear regression) and PLS (partial least square method) for correlating various physicochemical parameter with biological activity. SWF-PLS (model 3) was found to be the best model. The values of the regression coefficient, cross-validation coefficient and predicted r^2 indicated the best correlation and external predictive ability of the model.

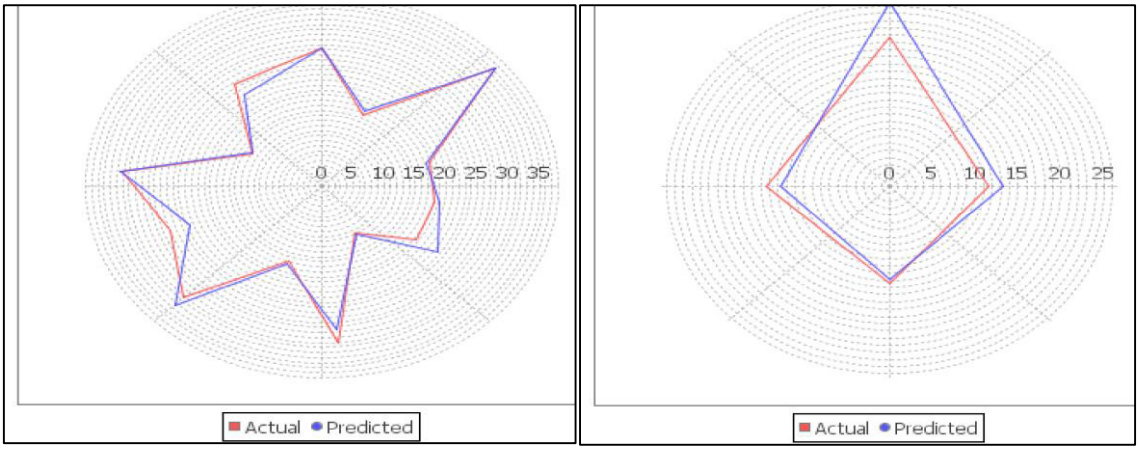
Table 3.2: The different descriptors with their significance and contribution towards the development of models generated.

Parameters	Significance	Contribution
XlogP	This descriptor exhibit the lipophilicity of the molecule	Positive
SsClE-index	Electrotopological state indices indicating number of chlorine atoms attached with one single bond.	Positive
Xcomp Dipole	exhibits the x component of the dipole moment (external coordinates).	Negative
Dipole moment	The dipole moment descriptor is calculated by counting the partial charges presented on the molecule	Negative
XKHydrophobic area	vdW surface descriptor showing hydrophobic surface area	Negative



(i) **(ii)**

Fig 3.1. (i) Contribution plot of various descriptors used in the development of 2D QSAR models; (ii) Fitness plot of training and test set molecules selected for the development of 2D QSAR models



(i) (ii)

Fig. 3.2. (i) Actual and predicted activity of test set indicated by overlapping of Radar plot (2D QSAR studies) (ii) Actual and predicted activity of training set indicating by overlapping of Radar plot (2D QSAR studies)

3.2 3D-QSAR

Number of 3D-QSAR models were generated using kNN-SWF, kNN-MLR variable selection method. The various parameters of developed models have been reported in Table 3.3. The various parameters of the generated models build a relationship between the experimental activity and molecular field analysis (MFA), including the hydrophobic (H) and electrostatic (E) fields, which were represented by red and blue colours, respectively, thus highlighting their effects on the biological activity. The Fig. 3.3 contributed various descriptors. The Radar plots for the actual and the predicted activities of the training sets and test sets were represented in Fig. 3.4.

Table 3.3. The different models generated for better correlation by statistical methods

Models	KNN-SWF (Model 1)	KNN-MLR (Model 2)
q^2	0.9294	0.8171
q^2_se	2.1154	4.3254
r^2	0.5978	0.7006
Pred_r^2se	10.5448	5.3387

The above table signifies the development of 3D QSAR models by following kNN –SWF statistical methods. The methods correlated various physicochemical parameter with biological activity. SWF-PLS (model 3) was found to be the best model. The values of regression coefficient, cross validation coefficient and standard error indicated the best correlation external predictive ability of the model.

3.2.1. kNN-MLR method

The kNN-MLR method was followed for the generation of significant models. The kNN method is based on the distance learning method. The distance between the unknown member and the nearest neighbours plays the major role in the calculation of the distance and thus their effect on the biological interactions. With the help of distance metric, the distance of the closest neighbours were calculated. The cross validation regression coefficient (q2=0.81) and the external cross validation coefficient (pred r^2= 0.70) values were found to be significant. The points generated in kNN-MLR method are H_630 (-0.0132 -0.0032) E_1965 (0.2682 0.7580) H_1382 (-0.3361 0.4018) E_859 (0.1934 0.4687) E_73 (-0.4646 -0.3085) which indicated that that the hydrophobic and electrostatic interactions are playing the major role in explaining the anti-inflammatory effectiveness. The ranges of the interactions are given in parenthesis.

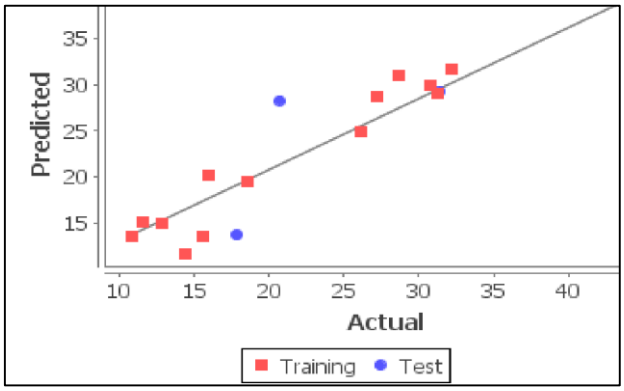
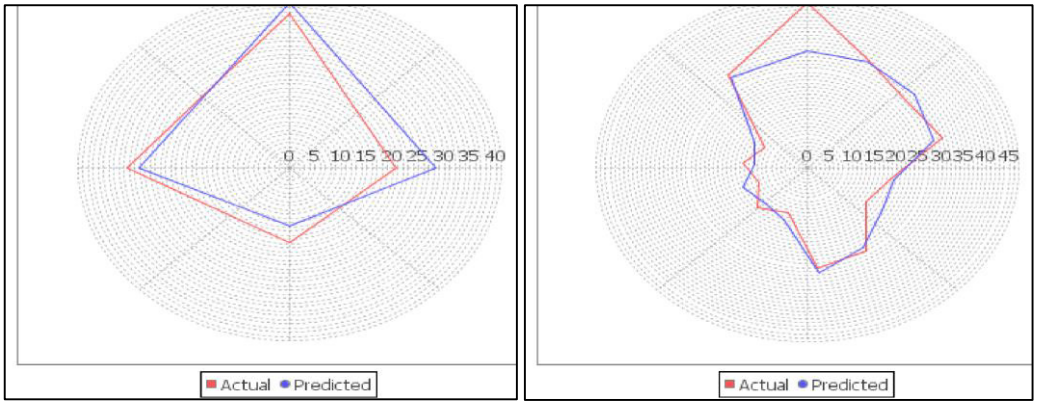


Fig. 3.3. Fitness plot of test and training set molecules selected for the development of 3D QSAR models.



(i) (ii)

Fig 3.4 (i) Actual and predicted activity of training set indicated by overlapping of Radar plot (3D QSAR studies) (ii) Actual and predicted activity of test set indicated by overlapping of Radar plot (3D QSAR studies)

3.3 Molecular Docking

The protein ligand interaction is the basis of molecular docking which helps in discovery and development of newer molecules. 29 hypothetical derivatives of 2-mercaptobenzothiazole was designed and docked against the protein TYrRS extracted from *S.aureus*. The binding interactions of the different amino acids: LYS84A, GLY83A, THR42A, SER85A, ARG88A in the active site were observed. The study followed the GRIP docking module, in which a set of prepared ligands along with its different conformers were docked against the protein molecule. A grid is generated by knowing about the cavity of receptor and ligand orientation requirements. This way, a theoretical picture of effective binding orientations was created. Further this helped in the documentation of the results regarding the best docked posture and the desired derivatives to be synthesized. The present study showed the binding orientation of the K6 compound, K20 compound, K18 compound into the active side of *S. aureus* TYrRS. (PDB ID:1JIJ) were found to be the more effective even than the standard drug Diclofenac sodium and the results were represented in Fig 3.5.

3.3.1 Docking Score

The scoring function of Vilfe MDS 4.6 docking program is represented as docking score. The docking score demonstrates the binding interactions of the ligands with the protein molecule. These mathematical representations are used to forecast the potency of the non-covalent interaction between two molecules.The binding affinity of the novel benzothiazole derivative to the protein is expressed in term of docking score. The docking score of standard compound (Diclofenac sodium) was found to be -28. The docking score of novel benzothiazole derivative K6, K18, K20 was found to be more the diclofenac sodium -48.52, -48.44, -47.46, and compound K20 having the highest docking score (Table 3.4). After deep analysis of the data, it was found that the docking score of all novel benzothiazole derivatives was comparable with the docking score of standard drug diclofenac sodium.

Table 3.4. Different docking parameters include D score, ligand pose, hydrophobic interactions and charge interactions involved with active amino acid residues.

Compound Number	Docking Score	Ligand Pose	Number of Residue	Hydrophobic Interaction	Hydrogen Bonding	Charge interaction
K6	-48.44	4	1	LYS84A	-	-
K18	-47.46	3	2	LYS84A,GLY83A	-	-
K20	-48.52	5	2	LYS84A,GLY83A	-	-
Diclofenac Sodium	-28.15	5	2	LYS88A	-	ARG88A

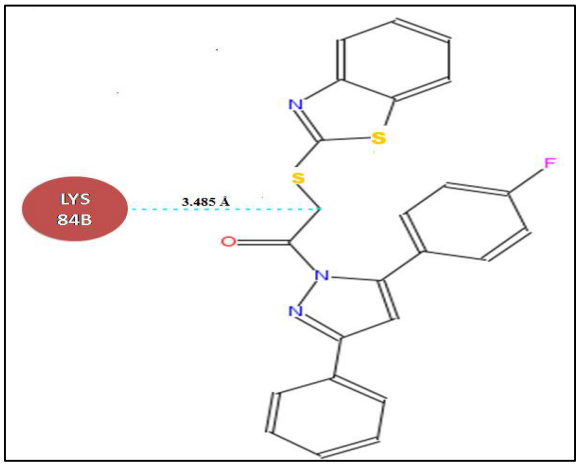


Fig (i)

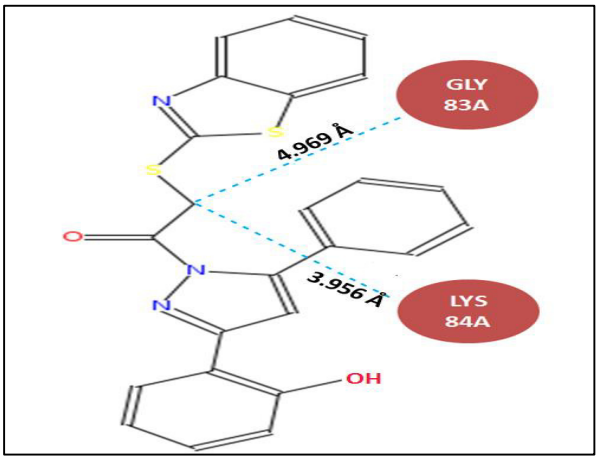


Fig (ii)

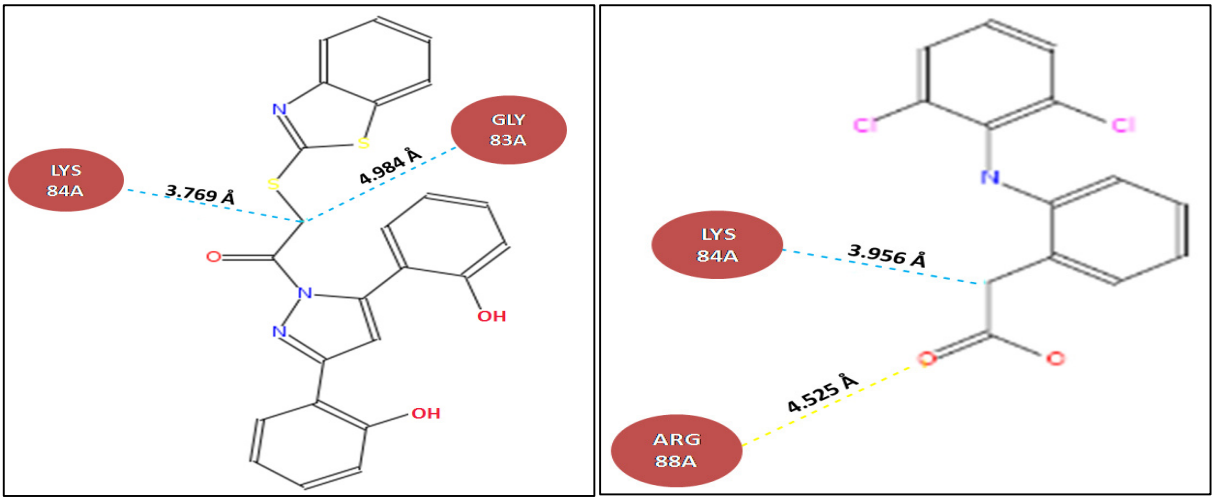


Fig (iii) Fig (iv)

Fig 3.5. 2D representation showing the binding orientation of the (i) K6 compound (ii) K20 compound (iii) K18 compound into the active side of *S. aureus* TYrRS. (PDB ID:1JIJ) (iv) Diclofenac sodium.

3.3.2 ADME Studies

A good interaction and docking score gives the idea about the binding of ligands with the protein and it does not imply solely that the ligands would be a suitable drug candidate. Pharmacokinetics of the drug also play an important role and the efficacy of a drug's passage through the body is evaluated by using parameters of absorption, delivery, metabolism, and elimination (ADME). Taking into consideration, a preliminary predictive *in silico* pharmacokinetic study of the selected three compounds was undertaken along with the reference drug as shown in the **Table 3.5.** Selected compounds under study are considered as a good drug candidate as they qualified required ADME parameters like cellular permeability (Caco), human intestinal absorption (HIA%), Madin- Darby canine kidney cells (MDCK), volume, Blood brain barrier (QPlogBB) etc . Further, drug likeness has been indicated by all molecules as per Lipinski rule with of five, which evaluate the molecules based on their molecular weight (Mol.wt.), partition coefficient (QPlogPo/w), hydrogen bond donor (DonorHB), hydrogen bond acceptor (AccptHB). Whereas MDDR rule accessed the probability of finding a 'druglike' compound based on no of rings ≥ 3, no. of rigid bonds ≥ 18, and no of rotatable bonds ≥ 6.

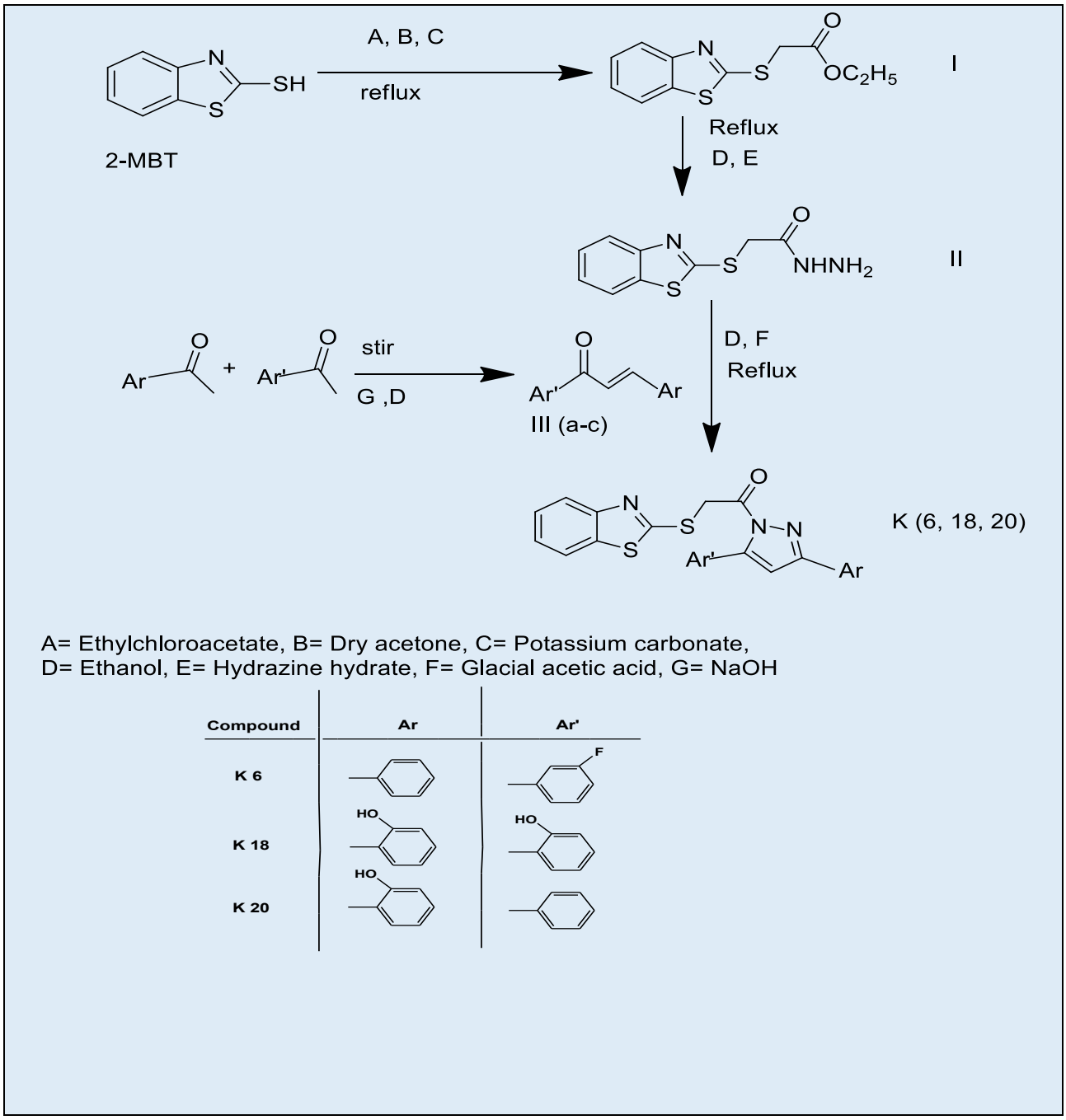
Table 3.5. Lipinski Rule of five, MDDR like rule and different ADME properties calculated for the K6, K18, K20 and the standard drug Diclofenac sodium

S. No.	PROPERTY	K6	K18	K20	DICLOFENAC SODIUM
1.	Lipinski Rule (Rule of five)	Suitable	Suitable	Suitable	Suitable
2.	MDDR like Rule	Mid-Structure	Mid-Structure	Mid-Structure	Mid-Structure
3.	BBB	1.66	0.05	0.55	0.69
4.	Caco2 cell membrane permeability	52.88	20.38	34.15	20.70
5.	HIA	97.74	96.13	97.091	96.14
6.	MDCK	0.17	0.06	4.39	58.33
7.	Plasma Protein Binding	94.11	93.45	93.99	100.00
8.	SKlogD value	7.16	6.64	6.83	3.08
9.	SKlogP value	7.16	6.64	6.83	4.33
10.	CYP 2D6 inhibition	Non	Non	Non	Non
11.	CYP 2D6 substrate	Non	Non	Non	Non
12.	CYP 3A4 inhibition	Inhibitor	Inhibitor	Inhibitor	Non
13.	CYP 3A4 substrate	Substrate	Weakly	Weakly	Weakly
14.	Skin Permeability	-2.35	-2.54	-2.28	-2.53
15.	Pgp inhibition	Inhibitor	Inhibitor	Inhibitor	Inhibitor

3.5 Chemistry

The target compounds were synthesized via Scheme I and the physicochemical data was represented in Table 3.6. The synthesized structures were found to be in accordance with the IR, ^{1}H NMR and ^{13}C NMR data. The ester derivative (Compound-I) and the hydrazide derivative (compound II) were obtained by following the method reported by Azam et al., 2010.[36] The ester derivative was obtained by refluxing 2-mercaptobenzothiazole with ethyl chloroacetate in dry acetone and confirmed by the presence of C=O stretch, at 1737.91 cm^{-1} and absence of peak around 2550 cm^{-1} of SH group. Then the Compound-I was refluxed with hydrazine hydrate in ethanol to obtained hydrazide derivative (compound-II) and formation of compound-II which was confirmed by the absence of ester C=O stretch, at 1737.91 $cm^{-1}$and presence of NH stretch at around 3285.52 cm^{-1} and C=O amide stretch at around 1646.73 cm^{-1}. Different

substituted chalcones were synthesized from substituted benzaldehyde and acetophenone and synthesis was confirmed by the presence of a stretch of ketone around 1650 cm^{-1} and stretch of different functional groups in each chalcone, which were then refluxed with hydrazide derivative (compound-II)in the presence of glacial acetic acid and dilute hydrochloric acid, maintain the pH up to 5.5 to 6.5 because more acidity would favour to the chalcone and more basicity would favor to hydrazide derivatives hence reaction occurs at pH 5.5 to 6.5 and synthesis was confirmed by the absence of NH stretch at 3285.52 cm^{-1} and presence of C=N stretch at around 1670 cm^{-1}respectively. Synthesis of final compounds was also confirmed by H^1NMR signals such as the peaks coming in the range of 6.883-6.989 ppm confirmed the proton of pyrazole ring and the protons of linker CH_2 showed a signal between the range 4.101-4.14 ppm respectively. The multiple peaks between the range 8.03-7.025 ppm confirmed the presence of aromatic protons. ^{13}CNMR signal for example 147.10 (C, pyrazole), 34.5 (C, CH_2) also confirmed the synthesis of final products.



Scheme I. Synthetic route followed for the synthesis of the 2-Mercaptobenzothiazole derivatives

Table 3.7. The physiochemical observations of synthesized compounds of 2-Mercaptobenzothiazole

S. No.	Compound	Rf	Solvent system	m.pt(^{0}C)	Yield in (%)	Mol. wt.
1	Compound-I	0.75	Hexane: ethyl acetate=8.5:1.5	41-44.	70.27	253.34
2	Compound-II	0.68	Methanol: Chloroform =1:9	161-166	76.25	239.02
3	3a	0.66	Ethylacetate: Hexane=2.5:7.5	92-96	66.07	226.08
4	3b	0.61	Ethylacetate: Hexane=2.5:7.5	80-88	68.05	240.25
5	3c	0.63	Ethylacetate: Hexane=2.5:7.5	89-95	61.12	224.25

3	K6	0.58	Ethylacetate: Hexane = 3:7	181-186	67.06	445.33
4	K18	0.53	Ethylacetate: Hexane =3:7	189-195	61.21	459.54
5	K20	0.49	Ethylacetate: Hexane =3:7	184-191	67.71	443.54

3.6 *In vitro* Anti-inflammatory Study

3.6.1 By Protein Denaturation Method

The *in vitro* study for the anti-inflammatory effect of the synthesized compounds was carried out against the denaturation of protein (egg albumin) at five different concentrations levels. The results of percentage inhibition and IC_{50} were recorded in Table 3.7. A concentration-dependent inhibition of protein (albumin) denaturation by compounds at different concentrations ranging from 62.50 µg/mL to 1000 µg/mL was observed. Diclofenac was used as a standard drug. It was observed that with the increase in concentration, the percentage inhibition increased (Table 3.7); however, the Diclofenac sodium was found to be less effective when compared with the synthesized compounds K6, K20. The results were further confirmed by observing their IC_{50} values. The compounds K6, K20, K18, compound I, compound II possessed 10.56, 5.99, 5.23, 45.70, 10.74 respectively. IC_{50} value whereas that of Diclofenac was found to be 12.78.

3.6.2 By HRBC Method

By following HRBC membrane stabilization method i*n vitro* anti-inflammatory activity was performed. The same observation was made that the activity increases as synthesized derivatives' concentration increased. So, it can be stated here that the *in vitro* anti-inflammatory activity is concentration-dependent. All the results were compared with standard Diclofenac sodium which showed 46.92% protection (Table 3.8). The percentage inhibition and $IC_{50\ results}$ of the synthesized compounds were calculated and displayed in Table 3.8. It was found that compounds K20, I, II, K18, were more potent and compound K6 exhibited comparable potential when compared with the reference drug Diclofenac sodium.

Table 3.7. Results of the *in vitro* anti-inflammatory activity by egg albumin method in terms of percentage inhibition

Compounds	Percentage inhibition at various concentrations					IC_{50} Values
	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.50 µg/ml	
Diclofenac sodium	49.35	10.56	38.70	32.98	14.02	12.78
K6	50.90	5.99	40.51	35.58	15.32	10.56
K18	48.57	5.23	39.22	42.33	14.54	5.99
K20	52.72	5.70	48.57	40.77	18.70	5.23
Compound I	40.00	10.74	37.92	32.98	10.12	5.70
Compound II	42.07	33.50	24.93	17.14	2.33	10.74

3.6.3 Structure-Activity Relationship (SAR)

From the above two *in-vitro* studies, synthesized 2-mercaptobenaothiazole derivatives have shown desired results as predicted by the molecular docking studies. Significant increase in anti-inflammatory activity was observed which was found to be comparable to the standard drug, Diclofenac sodium. Furthermore, all the tested compounds were found to be active, which may further be attributed to the presence of linker mercapto group ie. -SH and increase in anti-inflammatory activity was observed with derivatives bearing phenyl rings substituted with electronegative substitutions at *o*- and *p*-positions which were found to be similar to the results reported by Azam MA *et al.,* 2010.[36] In general, it can be stated that anti-inflammatory activity is dependent on the basic skeleton and the nature of the substituents present.

Table 3.8. Results of the *in vitro* anti-inflammatory activity by HRBC method in terms of percentage inhibition

Compounds	Percentage inhibition at various concentrations					IC_{50} Values
	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.50 µg/ml	
Diclofenac sodium	46.92	44.46	39.34	34.83	30.32	2.45
K6	52.04	45.28	40.98	38.93	33.60	3.74
K18	51.02	44.67	40.57	34.22	29.30	1.56
K20	56.78	41.36	43.85	37.09	31.55	1.28
I	45.08	42.41	38.31	31.14	26.02	1.57
II	39.34	34.83	25.81	30.32	21.92	1.28

4. CONCLUSION

In the present work, some novel derivatives of benzothiazole derivatives were designed and synthesized in appreciable yields. Firstly, 2D and 3D QSAR was carried out by using pIC_{50} of twenty-one derivatives of the benzothiazole from literature by using Vlife MDS 4.6. software. From the results of 2D and 3D QSAR hypothetical designing of some newer benzothiazole derivatives was carried out and their structures were drawn with the help of Chemdraw ultra 12.0. Vlife MDS 4.6 was used for optimizing the designed structures of all the 29 molecules along with the standard drug Diclofenac. They were energy minimized before the docking studies. The protein from *S. aureus* TYrRS. (PDB ID:1JIJ) was used and depending upon docking score some compounds were selected for synthesis. The synthesized compounds were evaluated for the *in vitro* anti-inflammatory activity, by using two methods i.e. egg albumin method and HRBC membrane stabilization method. It was observed that the derivatization of 2-mercaptobenzothiazole has increased anti-inflammatory

potential, evident from the results obtained. Compound K20 displayed potential anti-inflammatory activity which may be attributed to the electronegative group on the phenyl ring substituted on the pyrazole part. Even the compound K6 and K18 had shown effective IC_{50} compared to the standard drug Diclofenac sodium. The addition of pyrazole ring bearing substituted phenyl rings in the pharmacophore is responsible for the increased anti-inflammatory activity. And the presence of linker mercapto group (-SH) has also been equally significant for the activity.

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6. AUTHOR'S CONTRIBUTION STATEMENT

Mr. Krishan Kumar has carried out the experimental work related to the synthesis, *in vitro* biological evaluation and drafted the manuscript. Dr. Neelima Dhingra has performed the software studies and provided the related findings. Mr. Shammi Rajpal contributed towards the reviewing and writing of the manuscript. Dr. Archana Kapoor acted as supervisor and edited the final draft of manuscript. All authors have given approval to the final version of the manuscript.

7. CONFLICT OF INTEREST

There is no conflict of interest.

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