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Der Pharma Chemica, 2013, 5(6):53-61
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Virtual screening and computer aided analysis of 2,4,6-trisubstituted triazines as antimalarial agents

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ABSTRACT

The virtual screening carried out on the malarial inhibitors in search of generation of novel compound, till date no proper medication available for prophylactic of dangerous disease malarial. So in the seek of novel compounds three dimensional quantitative structure activity relationship analysis (3D-QSAR) performed using k nearest neighbor molecular field analysis (kNN MFA) method on a twenty 2,4,6-trisubstituted triazines derivatives as antimalarial agent by molecular design suite. The best model generated with 7.5 dissimilarity values with 2k and 2i as test set the statistical parameters were q^2 and $Predr^2$ for stepwise (0.8229 and 0.674), genetic algorithm (0.699 and 0.7521) and stimulated annealing (0.7069 and 0.6901) respectively. In virtual screening analysis the steric descriptor indicates that bulky groups were required for enhancing the activity at R position and on triazine ring, while the electrostatic groups suggested for attaching the electron donating and withdrawing groups at R position on triazine ring for enhancing the activity. Inhibition of the Tyr 108 leads to discontinue the metabolic process in protein, finally the enzyme fatal. The Tyr 108 of glutathione transferase interacts with morpholino ring. Thus structural requirement predicted by QSAR analysis and docking was used to design a noble compound.

Keywords: 3D-QSAR, kNN-MFA, Docking, antimalarial, 2,4,6-trisubstituted triazines derivatives

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INTRODUCTION

Malaria is airborne endemic disease which is cause by parasite, spreads in tropical and subtropical regions especially responsible for death and illness in children, it is estimated that about half of the world's healthy population lives in malaria widespread areas [1]. Malaria is dangerous disease spreads by protozoan of the genus *Plasmodium*, but in humans, four species responsible were as *P. falciparum, vivax, malariae* and *ovale* that are accountable for devastating disease. The several drugs were available in the bazaar but till date no significant prophylactic treatment of malaria. There were different problem related to enhancing malaria protozoa in living body like no proper choice of medication available, spreading of parasite was fast in the host body and most familiar was multi-drug resistance. The presented problem of multi-drug resistance to existing antimalarials drugs has lead to a major focus on the generation and optimization of new synthetic moiety [2]. There is very less data accessible on the essentiality of reputed drug targets for *Plasmodium* growth are limited to a few individual genes that have been tested in gene disruption studies [3-11]. The rational and emergence of multidrug resistance of the conventional antimalarial compounds has led to the need of the generation of new antimalarial drugs. Quantitative structure-activity relationship (QSAR) methods docking are used widely in the design and development of new antimalarial compounds [12-14]. QSAR attempts to correlate the structural/molecular properties in the form of descriptors (steric and electronic) with biological activities [15-19]. The virtual screening on 2,4,6-trisubstituted triazines derivatives

can be used to predict biological activity of compound and subsequently utilized in designing of novel antimalarial molecules.

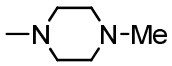
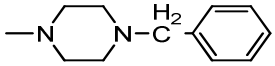
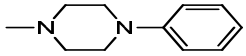
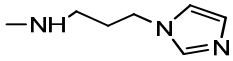
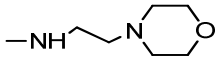
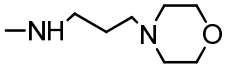
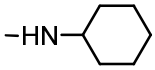
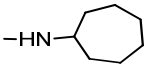
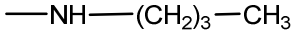
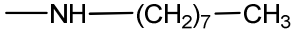
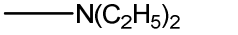
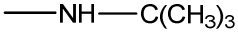
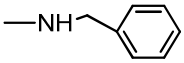
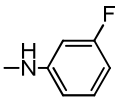
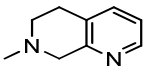
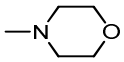
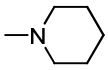
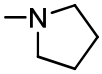
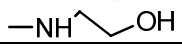
MATERIALS AND METHODS

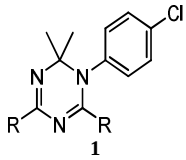
2.1 QSAR Analysis

2.1.1 Dataset

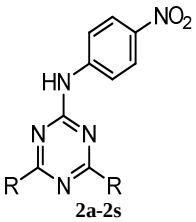
In the present study a dataset of 20 molecules of 2,4,6-trisubstituted triazines derivatives [20], has been taken from the previous literature for QSAR studies reported in table 1. The reported IC_{50} values (µM), have been converted to the pIC_{50}, for QSAR study. Sphere exclusion (SE) algorithm method used for separation of dataset into training and test set. Thus statistical analysis executed by kNN-MFA methodology with stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) was used for building the QSAR models.

Table 1 General structure of the compounds of 2,4,6-trisubstituted triazines derivatives and their biological activities (data set of 20 molecules)

Compound	R	IC_{50}(µM)	pIC_{50}
1	NH_2	64	4.1938
2a		1	6
2b		2	5.6989
2c		10	5
2d		1	6
2e		2	5.6989
2f		2	5.6989
2g		1	6
2h		2	5.6989
2i		2	5.6989
2j		10	5
2k		10	5
2l		50	4.301
2m		10	5
2n		10	5
2o		10	5
2p		10	5
2q		50	4.301
2r		50	4.301
2s		50	4.301



1



2a-2s

2.1.2 Molecular Modeling Analysis:

The structures were sketch on VlifeMDS sketch module software. The energy minimization and molecular modeling study was performed on selected dataset. The alignment was required for better analysis and generation of the promising significant model. The selected dataset were aligned using template based method by selecting most active molecule **2a** as a reference molecule (1) and structure (2) as a template shown in figure 1. The alignment of all the molecules on the template is shown in figure 1 as a reference aligned molecules. In the template based alignment method, benzene template structure was defined and used as a basis for alignment of a set of molecules. Once the molecules are aligned, a molecular field is computed on a grid of points in space around the molecule. Descriptors representing the steric, electrostatic and hydrophobic interaction energies were calculated at the lattice points of the grid via a methyl probe of charge +1. This field provides a description of how each molecule will be inclined in the active site.

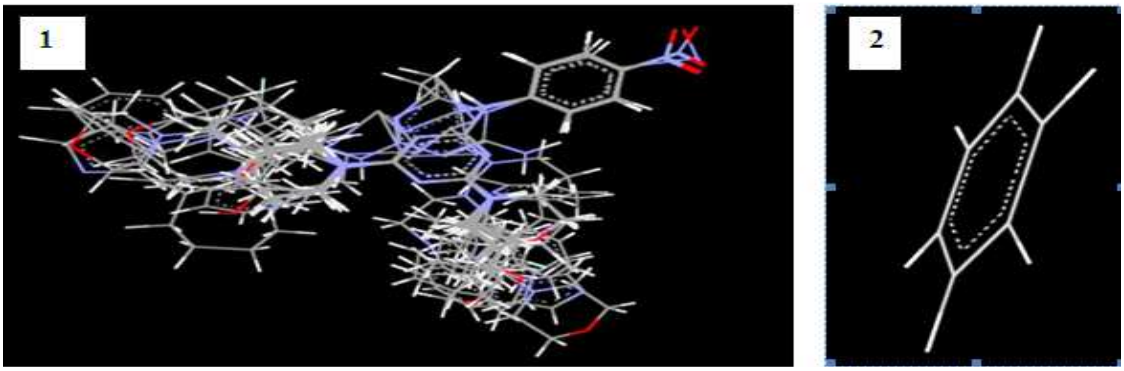


Figure 1 Template structure (1) and reference aligned structure (2) of malarial IV inhibitor

In order to evaluate the QSAR model externally and internally, data set was divided into training and test set using sphere exclusion methods. Training set is used to develop the QSAR model for which biological activity data are known [21]. The higher the dissimilarity level, the smaller the training set is and the larger the test set is and vice versa.

Test set is used to challenge the QSAR model developed based on the training set to assess the predictive effectiveness of the model which is not included in model generation. Different training and test set of 2,4,6-trisubstituted triazines derivatives were constructed using sphere exclusion with dissimilarity level 7.5 to 11. Training and test set were selected and calculated unicolumn statistics. Unicolumn statistics suggested that maximum of training should more than test set and test of minimum should be more than training set. As unicolumn statistics adjusted with dataset further analysis was performed.

The generation of statistical model depends on method which is selected for analysis. Data generated by k nearest neighbor molecular field analysis (kNN-MFA) in conjunction with stepwise (SW), forward-backward, simulated annealing (SA) and genetic algorithm (GA) variable selection methods with pIC50 activity field as dependent variable and descriptors as independent variable [22-25]. The stepwise (SW) forward-backward, simulated annealing (SA) and genetic algorithm (GA) method utilized for exploring the statistical parameters. The several models are generated with contour plot by selecting different training and test set, cross validation correlation coefficient (q^2) and good predictivity (pred r^2) shows effective finding.

2.2 Docking analysis

The chemical structure were constructed on the chemdraw ultra 8.0 and transformed to 3 dimensional (3D) structure by chem 3D ultra 8.0 and energy minimization by MOPAC and MM2 done. The 3D structures were saved in .mol format for docking analysis. Docking binding energy and binding site was detected by the molegro 5.0 software. The pdb of *plasmodium falciparum* downloaded from pdb site. Docking analysis completed in three steps, firstly importing the pdb in the workspace of molegro 5.0, preparation of it performed as removal of water molecule, cofactor and ligand attached to protein. The surface area of protein developed and subsequently detection of cavity done. The five cavities were detected as default in molegro 5.0. Secondly the structures were imported in the

workspace of the molegro and preparation of structure created. Finally the docking wizard analysis started to generate the binding affinity data in the form of binding energy as dock score and binding interaction [3].

RESULTS AND DISCUSSION

3.1 QSAR interpretation

The virtual screening and correlation of biological activity profile with in the data set gives a hint for designing of active compound which combat with existing malarial disease. The activity of the compound divided on the basis of inhibitory concentration of *Plasmodium*. The highly potent compounds 2g, 2d and 2a (IC_{50}= 1 µM) contain 1-amino-cyclohexane, 3-(1H-imidazol-1-yl)-N-propan-1-amine and 4-methylpiperazine. Clue indicates that amino and nitrogen containing ring increases the activity of the compounds. Slight decrease in activity achieved in compounds 2b, 2e, 2f, 2h and 2i (IC_{50}= 1 µM) have 4-benzyl-piperazine, 1-morpholinoethanamine, 1-morpholinopropanamine, N-cycloheptanamine and butyl amine respectively. Due to presence of aliphatic chain activity of molecule decreased in comparison to parent analogues.

Moderate activity was found in compound 2c, 2j, 2k, 2m, 2n, 2o and 2p (IC_{50}= 10 µM). The following compounds hold the functional group at R as 4-phenylpiperazine, octylamine, diethylamine, 1-benzylamine, 3-fluor-benzenamine, 5,6,7,8-tetrahydro-1,7-naphthyridine and morpholine respectively. Halogen and long aliphatic chain reduces biological activity as compared to parent analogues.

Least activity (IC_{50}= 50 µM) of 2l, 2q, 2r and 2s have 2-methylpropan-2-amine, 1-piperidine, 1-pyrrolidine and 1-aminoethanol at R. The saturated compound reduces the activity of compounds. Poor activity was found with compound 1 which have amino group on R. The activity of novel compound can be enhancing by attaching hetro aromatic ring with nitrogen containing ring.

The selected series consist of 20 compounds for performing the QSAR studies the dataset was divide into the test and the training set. Different training and test set of 2,4,6-trisubstituted triazines derivatives were developed using sphere exclusion (dissimilarity level 7.5 to 11). The training and test set were selected, on the basis of Uni-column statistics, i.e., maximum of the test is less than maximum of training set and minimum of the test set is greater than of training set, which is prerequisite for further QSAR analysis shown in table 2.

Table 2 Uni-Column Statistics for Model 1 for training and test set activity.

Column Name	Average	Max	Min	Std Dev	Sum
Training set	5.1995	6.0000	4.1938	0.6344	93.5913
Test set	4.6505	5.0000	4.3010	0.4943	9.3010

The best model 1 showed that steric (S_402) and electrostatic (E_1072) interactions engage in recreation role in determining antimalarial activity. This analysis provide insight that the test is interpolative i.e., derived from the minimum and maximum range of training set. The mean and standard deviation of the training and test set provides insight to the relative difference of mean and point density distribution of the two sets. k-Nearest neighbor molecular field analysis (kNN-MFA) was applied using stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) approaches for building QSAR models. Results of models developed by SW-kNN MFA, SA-kNN MFA and GA-kNN MFA using sphere exclusion methods. Significant QSAR model generated is shown in table 3.

Table 3 Result of kNN-MFA study using sphere exclusion selection method

Model	DV	Test set	SW-kNN MFA		GA-kNN MFA		SA-kNN MFA	
			q2	Predr2	q2	Predr2	q2	Predr2
1	**7.5**	**2k, 2i**	**0.8229**	**0.674**	**0.699**	**0.7521**	**0.7069**	**0.6901**
2	8	2k, 2i, 2q	0.6554	0.6712	0.6801	0.4495	0.5908	0.4516
3	10	2g, 2k, 2i,2q	0.6103	0.4973	0.5136	0.4194	0.6191	0.6447
4	11	2p, 2i, 2k, 2l, 2q	0.6962	0.7624	0.6355	0.4302	0.6751	0.7107

DV: Dissimilarity value

The uni-column statistical parameter where useful for suitable selection of data set for systemic QSAR analysis The uni-column parameter as average, maximum, minimum and standard deviation provides valuable suggestion regarding the analysis of QSAR model generation. If the maximum of the training set more as compared to test and minimum of test set is more as compared to the training set then the dataset was accepted for consequent analysis. In

analysis the standard deviation must be least to determine the statistically consistent parameters. The value generation after uni-column studies helps better understanding of structural requirement in the compound.

The dissimilarity values range from 7.5 to 11 influences the statistical parameter. The different model generated by changing dissimilarity value on the basis of these, the dataset in term of training set and test set fluctuated which helps in development of best model. The best statistical model parameters were utilized to correlate the biological activity with the structural requirement. The best model generated with 7.5 dissimilarity values with 2k and 2i as test set the statistical parameters were q^2 and $Predr^2$ for stepwise (0.8229 and 0.674), genetic algorithm (0.699 and 0.7521) and stimulated annealing (0.7069 and 0.6901).

The IC_{50} of the compound ranges from 64 to 1 and pIC_{50} from 4.1938 (1) to 6 (2a, 2d, 2g) respectively. The most active compound 2a, 2d and 2g presented good evidence, has similar biological activity, consist of methyl piperazine, 1-amino-propyl-imidazole and 1-amino-cyclohexyl at R respectively. The stepwise (5.67473 to 4.47005), genetic algorithm (5.83722 to 4.60167) and stimulated annealing (5.77571 to 4.19812) the predicted value found were 5.67473, 5.83722 and 5.77571 for most active compound (2h, 2e and 2p) with functional group cycloheptyl amine, 1 amino ethyl morpholine and 1 morpholine at R respectively according to analysis. In the analysis it was found that if the at R position attached with morpholine ring the activity of compound remain maintain as the amino and other steric group added the activity increased. In stepwise, genetic algorithm and stimulated annealing minimum predicted activity were found with compound 2k, 2h and 2s as 4.47005, 4.60167 and 4.09812 which contains the diethylamine, cycloheptylamine and hydroxyl ethylamine at R position. Analysis suggested that if the straight chain or aliphatic ring changed with aromatic nitrogen and oxygen containing ring the biological activity definitely increased.

The residual value is the difference between the actual and predicted Pic50 of the model. The different methods as stepwise (1.17174 to -0.09996), genetic algorithm (1.13554 to -0.82785) and stimulated annealing (0.82793 to -0.77571) generated the predicted value and residual value for model 1. The residual value and predicted value of the model 1 presented on the table 4 gives an idea about the changes in biological activity within the dataset. In stepwise, genetic algorithm and stimulated annealing residual value maximum fluctuation found were 1.17174, 1.13554 and 0.82793 for compound 2g, 2g, 2r as functional group cyclohexylamine, cyclohexylamine and butylamine at R position. The activity least changed in comparison to actual pIC50 for predicted pIC50 were compound 2h (0.02417), 2j (0.1469) and 2j (0.02555) with functional group cycloheptylamine, octylamine and octylamine at R position.

Table 4 Actual and predicted biological activity for Training set and test set for model 1.

Compound	Actual IC_{50}	Predicted pIC_{50} SW-KNN MFA	Residual SW-KNN MFA	Predicted pIC_{50} GA-KNN MFA	Residual GA-KNN MFA	Predicted pIC_{50} SA-KNN MFA	Residual SA-KNN MFA
1	4.1938	4.47574	-0.28194	5.02165	-0.82785	4.30566	-0.11186
2a	6	5.59946	0.40054	5.19579	0.80421	5.51997	0.48003
2b	5.6989	5.42472	0.27418	5.24203	0.45687	5.59877	0.10013
2c	5	5.59945	-0.59945	4.68638	0.31362	5.60117	-0.60117
2d	6	5.59944	0.40056	5.68881	0.31119	5.52388	0.47612
2e	5.6989	5.67472	0.02418	5.83722	-0.13832	5.59753	0.10137
2f	5.6989	5.42472	0.27418	5.83722	-0.13832	5.60043	0.09847
2g	6	4.82826	1.17174	4.86446	1.13554	5.29173	0.70827
2h	5.6989	5.67473	0.02417	4.60167	1.09723	5.59773	0.10117
2i	5.6989	4.82826	0.87064	4.81874	0.88016	4.87097	0.82793
2j	5	5.34947	-0.34947	4.8531	0.1469	4.97445	0.02555
2k	5	4.47005	0.52995	4.82763	0.17237	4.45062	0.54938
2l	4.301	4.65049	-0.34949	5.04558	-0.74458	4.3094	-0.0084
2m	5	5.59946	-0.59946	5.54589	-0.54589	4.9689	0.0311
2n	5	4.65051	0.34949	5.41762	-0.41762	5.77406	-0.77406
2o	5	4.65051	0.34949	4.68695	0.31305	4.6237	0.3763
2p	5	4.65051	0.34949	4.65681	0.34319	5.77571	-0.77571
2q	4.301	4.82826	-0.52726	4.82672	-0.52572	4.79665	-0.49565
2r	4.301	4.65049	-0.34949	5.02287	-0.72187	4.33112	-0.03012
2s	4.301	4.82525	-0.52425	4.9478	-0.6468	4.19812	0.10288

Statistical measures used are shown in table 4 to correlate biological activity and molecular descriptors. Data fitness plot for model is shown in figure 2. Result of the observed and predicted biological activity for the training and test compounds for the Model is shown in table 4. The plot of observed vs. predicted activity of training and test sets for

model is shown in figure 3. From the plot it can be seen that kNN-MFA model is able to predict the activity of training set quite well (all points are close to regression line) as well as external. Sphere exclusion (SE) algorithm and random selection methods were used for constructing training and test sets. kNN-MFA methodology with stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) was used for building the QSAR models and alignment molecule with descriptor shown in figure 4. The kNNMFA contour plot (figure 5) provided further understanding of the relationship between structural features of 2,4,6-trisubstituted triazines derivatives and their activities which should be applicable to design newer potential as antimalarial agent.

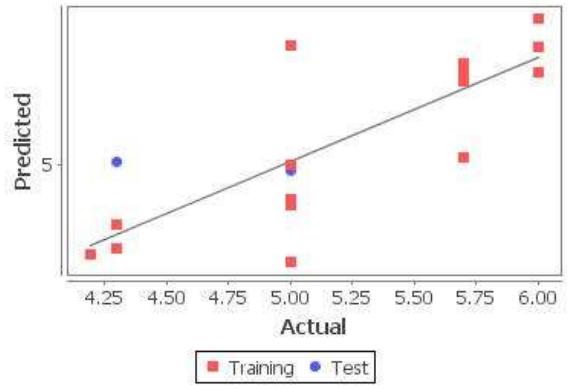


Figure 2 Graphical fitness plot between actual and predicted activity values as antimalarial agents for training and test set

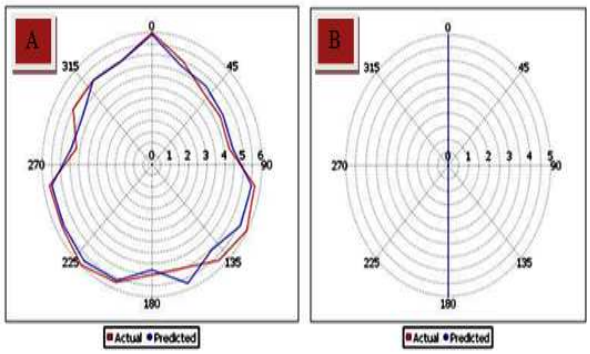


Figure 3 Training set (A) and Test set (B) biological activity is predicted graph

In contour maps the steric descriptor were positive S_1416 (30.0000 30.0000), S_659 (0.0477 0.5012), while the electrostatic descriptor were negative E_919 (-0.1480 -0.1236) for the significant model. In analysis the steric descriptor indicates that bulky groups were required for enhancing the activity at R position on triazine ring, while the electrostatic groups suggested for attaching the electron donating groups at R position and on triazine ring.

Statistical measures used are shown in table 4 to correlate biological activity and molecular descriptors. Data fitness plot for model is shown in figure 2. Result of the observed and predicted biological activity for the training and test compounds for the Model is shown in table 4. The plot of observed vs. predicted activity of training and test sets for model is shown in figure 3. From the plot it can be seen that kNN-MFA model is able to predict the activity of training set quite well (all points are close to regression line) as well as external. Sphere exclusion (SE) algorithm and random selection methods were used for constructing training and test sets. kNN-MFA methodology with stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) was used for building the QSAR models and alignment molecule with descriptor shown in figure 4. The kNNMFA contour plot (figure 5) provided further understanding of the relationship between structural features of 2,4,6-trisubstituted triazines derivatives and their activities which should be applicable to design newer potential as antimalarial agent.

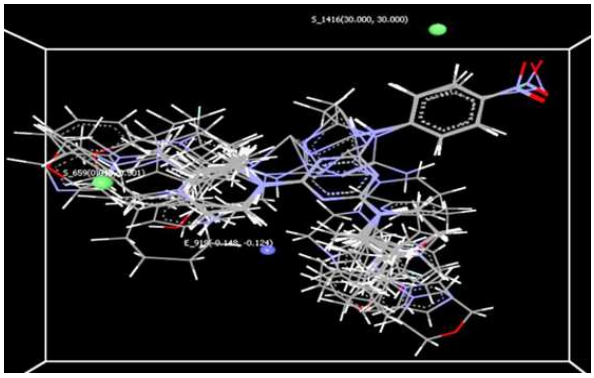


Figure 4 3D alignments of molecules and descriptor of model by wire frame model

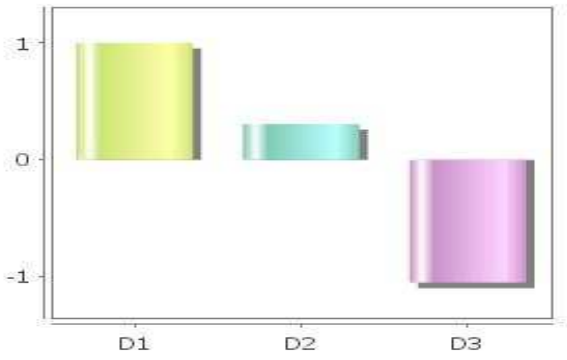


Figure 5 The kNN-MFA contour plots shows structural features of derivatives and their activities.

3.2 Docking interpretation

The docking study performed on pdb 3FR6 of *plasmodium falciparum*. The protein glutathione transferase selected for docking studies with 20 molecules. The docking studies required the preparation of protein and the molecule for stability and minimization of error. After preparation of the protein surface area determines. On the created surface area 5 cavities as default selected. The cavity was found with volume and surface. The volume and surface of cavities were predicted assigning as cavity 1 (130.56 and 568.32), cavity 2 (70.656 and 267.52), cavity 3 (51.2 and 207.36), cavity 4 (31.744 and 140.8) and cavity 5 (29.69 and 112.64). The protein and molecule subsequently imported on the workspace to start the docking wizard to generate the moldock score reported in the table 5. On the basis of moldock score active molecule can be detected. The maximum moldock score was found 114.47 for compound 2p.

Table 5 The binding affinity in the form of moldock score, rerank score and H bond of significant compounds (2p, 2f, 2e, 2c and 2b) presented

Code	Moldock	Rerank Score	H Bond
2p	-114.437	-100.78	-6.99
2f	-111.453	-85.89	-8.61
2e	-110.888	67.91	-8.06
2c	-103.113	62.51	-10.1
2b	-101.388	88.14	-6.83

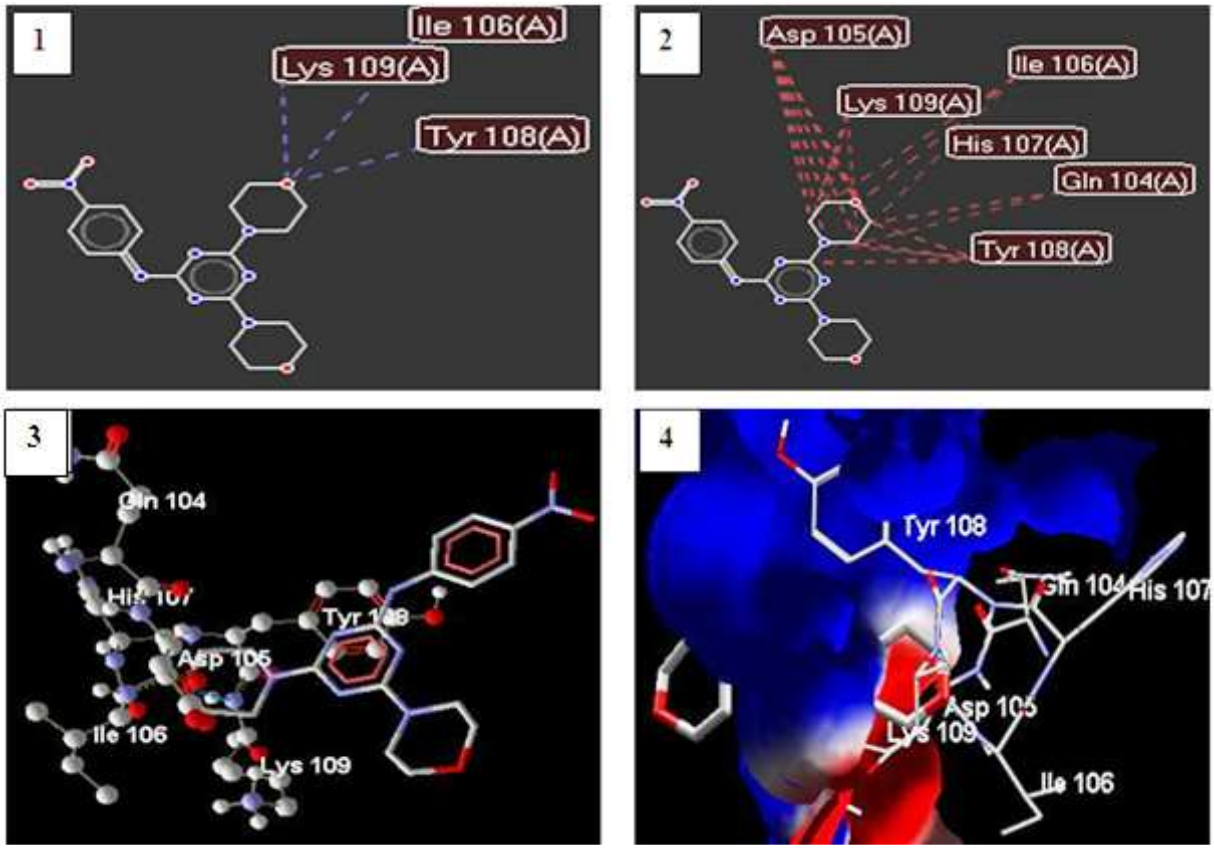


Figure 6. The docking binding affinity of 2p molecule presented as hydrogen bond interaction (1), hydrophobic interaction (2), 3D hydrogen bond interaction (3) and 3D hydrophobic interaction (4) with enzyme glutathione transferase (3FR6)

The hydrogen bond and the hydrophobic interaction of molecule 2p shown in the figure 6, helps in the interpretation and analysis of structural requirement of designing a novel compound. At R position of 2, 4, 6 triazine ring as morpholino group present which shows hydrogen bond interaction affinity with the Lys 109, Ile 106 and Tyr 108. In morpholino nitrogen and oxy atoms present. Oxy atom of morpholino ring creates hydrogen bond acceptor interaction with amine of glutathione transferase. The hydrophobic interaction of Asp 105, Ile 106, Lys 109, His

107, Gln 104, Tyr 108 was establish with morpholino and triazine ring. The Tyr 108 was responsible for survival of the enzyme glutathione transferase. Thus inhibition of the Tyr 108 leads to discontinue the metabolic process in protein, finally the enzyme fatal. The Tyr 108 of glutathione transferase interacts with morpholino ring (figure 6). On the basis of the docking study got indication that the morpholino ring was responsible for enhancing the biological activity. The docking binding affinity presented as (figure 6) hydrogen bond interaction (1), hydrophobic interaction (2), 3D hydrogen bond interaction (3) and 3D hydrophobic interaction with enzyme glutathione transferase (3FR6).

3.3 Virtual screening

The purpose of this study was not only to developed model, contour and to predict the estimated activity of the compounds, but also to employ the hypothesis on virtual screening to search novel scaffolds. In virtual screening analysis, we have built 3D QSAR models on malarial parasite inhibitors, the best quantitative model selected and by the help of docking features the active site of enzyme and active functional group determined. The reflected features of antimalarial could be used as fast and accurate tool to assist discovery of novel inhibitors. The designed scaffold used in further drug discovery of malarial inhibitors Figure 7.

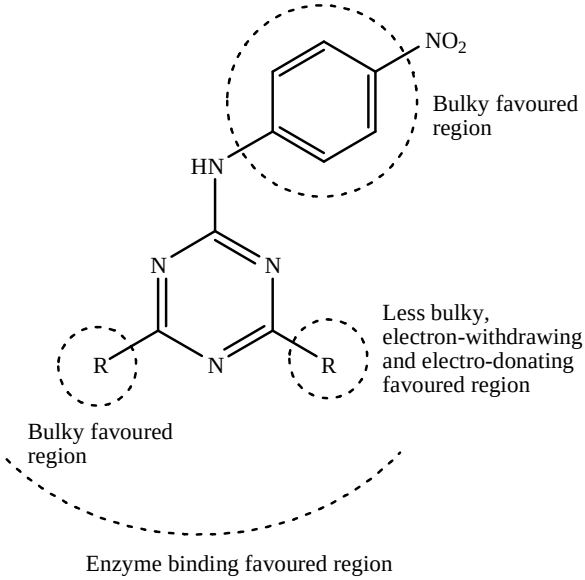


Figure 7 The designed scaffold used in drug discovery of malarial inhibitors through virtual screening

CONCLUSION

The dataset were divided into test and training set to developed the significant model in sphere exclusion data selection method. Model developed with statistical parameter to predict the structural requirement of 2,4,6-trisubstituted triazines derivatives as antimalarial agents reveals useful information about the structural features requirement for the molecule. In analysis the steric descriptor indicates that bulky groups were required for enhancing the activity at R position and on triazine ring, while the electrostatic groups suggested for attaching the electron donating and withdrawing groups at R position and on triazine ring. In further analysis it was found that if R position attached with morpholine ring the activity of compound remain maintain as the amino and other steric group added the activity increased. Inhibition of the Tyr 108 leads to discontinue the metabolic process in protein, finally the enzyme fatal. The Tyr 108 of glutathione transferase interacts with morpholino ring. On the basis of steric and electrostatic parameter the developed model in this work is useful in describing efficiency of QSAR on selected 2,4,6-trisubstituted triazines derivatives as antimalarial activity and further docking analysis can be employed to design new derivatives with potent inhibitory activity.

Acknowledgements

The authors are thankful to the Head, School of Pharmacy for providing facilities and to V-Life Science Technologies Pvt. Ltd. (Aundh, Pune, India) for providing the software. Swaraj Patil is grateful to the University Grants Commission, New Delhi for fellowship.

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