

Design, Synthesis and Evaluation of Pyrido [1,2] a benzimidazoles derivatives as Anti-malarial Efficacy**Deshbandhu Joshi¹, Ekta Sainani², Pooja Kumari³**¹**Professor, Department of Pharmaceutical Chemistry, Shrinathji Institute of Pharmacy, Nathdwara, Rajsamand, Rajasthan INDIA**²**Assiatant Professor, Department of Pharmaceutical Chemistry, Shrinathji Institute of Pharmacy, Nathdwara, Rajsamand, Rajasthan INDIA**³**Research Scholar, Department of Pharmaceutical Chemistry, Shrinathji Institute of Pharmacy, Nathdwara, Rajsamand, Rajasthan INDIA****Article Info: Received: 11-08-2026 / Revised: 16-09-2026 / Accepted: 04-10-2026****Correspondence: Deshbandhu Joshi****Conflict of interest statement: No conflict of interest****Abstract**

Quantitative structure–activity relationship (QSAR) analysis and molecular docking were conducted on 40 benzimidazole analogues to investigate their antimalarial potential. A dataset comprising 32 training compounds was used to develop comparative molecular field analysis (CoMFA), comparative molecular similarity indices analysis (CoMSIA), and hologram QSAR (HQSAR) models. The developed models demonstrated promising statistical performance, with reported predictive (r^2) values of 0.882, 0.788, and 0.867 for CoMFA, CoMSIA, and HQSAR, respectively. Molecular docking was additionally performed against the *Plasmodium falciparum* protein structure identified by PDB ID 2ANL to investigate the potential binding interactions of the designed compounds within the target binding site. Guided by the computational findings, 15 styryl benzimidazole derivatives were designed, synthesized, structurally characterized, and evaluated for their antimalarial activity. The experimental activity data were subsequently examined using CoMFA and CoMSIA analyses to assess the relationship between molecular features and biological activity. Collectively, these computational and experimental investigations provide a basis for identifying promising benzimidazole derivatives for further antimalarial drug development.

Keywords: CoMFA; CoMSIA; HQSAR and docking; studies as Plasmodium falciparum.**Introduction**

Malaria is an infectious disease caused by a blood-borne protozoan of the genus *Plasmodium* (P) and is transmitted by the female *Anopheles* mosquito. Among the more than 120 species from the genus *Plasmodium* five are currently known to infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. knowlesi* and *P. ovale* [1]. The most important of these in term of mortality is *P. falciparum*, although *P. vivax* also has a huge impact on population with regard to morbidity. According to the World Health Organization report 2017, there were an estimated 445000 deaths from malaria globally [2]. To overcome the failures of mono-therapy

and to address the different underlying defects of the pathology of malaria, modern drugs are effectively being used, either singly or in combination, as adjuncts to dietary therapeutic measures. Heterocyclic compounds are acquiring more importance in recent years because of their pharmacological activities. The imidazole nucleus is an important synthetic strategy in drug discovery. Analysis of scientific papers in the last two decades revealed that there is a general trend in research for new drugs involving modification of existing biologically active matrices and molecular design of the structures of

compounds. The imidazoles nucleus is an important synthetic strategy in drug discovery. Imidazole derivatives exhibited anti-plasmodial, antimicrobial, anti-inflammatory, analgesic, anti-tubercular and anticancer activity. To counter this, a range of newer drugs and combinations have gradually been introduced into use, e.g., mefloquine (1984), artemisinins (1994), artemether/lumefantrine (1999), atovaquone/proguanil (1999), chlorproguanil/dapsone (2003), but all come with some issue limiting use. There is thus a clear need to develop new more affordable and effective antimalarials. The presence of the benzimidazole ring ensures the promising anti malarial activity [3]. By implicating in silico designing approaches to a series of the nucleus, there is an enormous possibility to develop some novel promising entities. Quantitative structure activity relationship (QSAR) is used as a commanding tool for assessment of contribution of different fragment and properties of molecules with biological activity. Several attempts are made previously to establish the relation between structure of benzimidazoles nucleus and its reducing potential. In 3d QSAR (CoMFA and CoMSIA) different descriptor (steric, electrostatic, hydrophobic, donor and acceptor) used for statistical model generation. HQSAR fragment distinction map also shows good statistical relation between structure and biological activity. Docking studies reveals the interaction of ligand to the active site of protein. This helps us to evaluate enzyme ligand interactions at the molecular level with different amino acid bindings necessary for physiological response. Our current efforts are to spot light on the 3d QSAR (CoMFA and CoMSIA), 2d QSAR (HQSAR) and molecular docking studies for designing, synthesis, characterization and evaluation of novel benzimidazoles derivatives for anti malarial activity. A total fifteen

compounds were tested for anti malarial activity on *Plasmodium falciparum* strain. The synthesized compounds were confirmed by the FTIR, ^1H NMR, ^{13}C NMR, Mass spectra and other analytical data.

Material and methods

A selected compounds series of 40 Pyrido [1,2] a benzimidazoles were developed by Ndakala, A et. Al [3]. The IC_{50} (concentration of an inhibitor is reduced by half) and compounds of this series are shown in the Table-1. The effective concentration values (IC_{50}) was converted to negative log from (pIC_{50}) and used for QSAR model generation. CoMFA, CoMSIA and HQSAR models were generated using 8 compounds in test set and 32 compounds in training set. All the synthesized compounds melting point were determined using Melting point apparatus. Mass spectra of all the compounds were obtained on waters Q-TOF micromass (ESI-MS) at Sophisticated Analytical Instrumentation Facility (SAIF), panjab University, Chandigarh. The ^1H NMR and ^{13}C NMR spectra of all the compounds were obtained on Bruker Advance II 400 NMR spectrometer at SAIF, Panjab University, Chandigarh. The IR spectrum of compounds were determined using FTIR. All the synthesized compounds were screened for anti malarial activity in the Microcare laboratory & TRC, Surat, Gujarat.

Structural alignment

All the compounds were energy minimized using Tripos force field and Gasteiger Huckel charge in distance dependent dielectric and conjugate gradient method with convergence criterion 0.01kcal/mol. CoMFA, CoMSIA and HQSAR models were generated on SYBYL X 2.1 and docking studies was performed on SYBYL X 2.0 and Molegro Virtual Docker version 5.

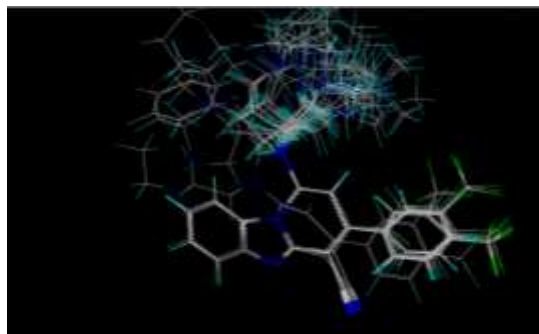


Fig.1- Structural alignment of compounds

CoMFA

The aligned training set of molecules was positioned inside grid boxes with grid spacing value of 2Å (default distance) in all Cartesian direction and CoMFA steric and electrostatic fields were calculated. The interaction energies for each molecule were calculated at each grid point using the (vdW interaction) and electrostatic (coulombic values) fields were calculated at each intersection on the regularly spaced grid. The cutoff value for both steric and electrostatic interaction was set to 30Kcal/mol. Different charges Gasteiger, Gasteiger–Huckel, Delre, Pullman, MMFF94 and Formal charges were used to generate the CoMFA models. The best CoMFA model was generated on MMFF 94 charge. PLS analysis was performed with cross validation (LOO) and then using no validation with a column filtering of 2.0. The quality of the CoMFA models can be evaluated by the obtained q^2 and the predictive capability of the models can be determined by r^2_{pred} . The predicted activities for the test set were obtained from the model produced by the training set [5].

CoMSIA

CoMSIA qsar method overcomes the deficiencies of Lennard- Jones and Coulomb potentials which are used in CoMFA method because it uses Gaussian function to calculate the interaction energy between compounds and probe atom. Steric, electrostatic, hydrophobic, H-bond donor and H- bond acceptor fields are used in CoMSIA analysis for charge calculation. The CoMSIA models were generated using a sp³ hybridized carbon atom having +1 charge, attenuation factor 0.3 and van der Waals radius of 1.4 Å. This probe atom is placed in every point of the lattice to calculate to calculate the different CoMSIA field which

are steric, electrostatic, hydrophobic, H-bond donors and H-bond acceptor fields. The different CoMSIA models gave highest statistical value in SEHD (steric, electrostatic, hydrophobic, and donor field) combination at MMFF94 charge. CoMSIA employs Gaussian type distance dependence and similarity indices for the generation of clear and smooth contour maps [6]. The final CoMSIA model was generated with highest cross validated r^2 (q^2) and convention r^2 with optimum number of component.

HQSAR

HQSAR is a new 2D-QSAR technique which employs specialized fragment fingerprints as predictive variables of biological activity. HQSAR does not require 3D alignment for model generation and is sensitive to three parameters concerning hologram generation, including hologram length, fragment size, and fragment distinction. The fragments distinct are atoms (A), bonds (B), connections (C), hydrogen atoms (H), chirality (Ch), and donor (D). Initially, various models were developed by using the default fragment size of 4 to 7 and different component, then based on the different fragment distinctions determined by the first step, the models were developed using different fragment sizes. The models with better result were applied to different fragment size and component number [7]. The better statistical results were obtained in fragment size 2 to 5, A/B/Ch/D distinct and number of component 6.

Docking analysis

Molecular docking studies was carried out using the Surflex Dock module in SYBYL X2.0 software. The protein structure of pdb name along with their inhibitor was retrieved from RCSB Protein Data Bank (PDB entry code:

2ANL). The protein structure was subjected to energy minimization and charge calculation (AMBER7FF99). After that the known complex Protein structure was used to investigate and validate the docking protocol. All ligands and

water molecules were removed. The bloat values was set as 1 and the threshold values as 0.5 for generation of protomol and position was considered to be the active sites for potential receptor's binding sites [8].

Table-1. Residual values of HQSAR, CoMFA, CoMSIA of training & test set

S. No.	Com. No	HQSAR A/B/DA			CoMFA		CoMSIA	
		Actual pIC ₅₀	Predicted pIC ₅₀	Residual value	Predicted pIC ₅₀	Residual Value	Predicted pIC ₅₀	Residual Value
1	1	5.7447	5.8299	-0.0852	5.8462	-0.1015	5.7376	0.0071
2	2	5.7447	5.8047	-0.06	5.7911	-0.0464	5.7793	-0.034
3	3	6.0809	6.058	0.0229	6.0375	0.0434	6.0477	0.0332
4	4	5.8623	5.881	-0.0187	5.8571	0.0052	5.8555	0.0068
5	5	5.5952	5.5848	0.0104	5.6557	-0.0605	5.6211	-0.025
6	6	5.5952	5.6092	-0.014	5.5864	0.0088	5.596	-0.008
7	7	5.8297	5.8653	-0.035	5.8694	-0.0397	5.8421	-0.0125
8	8	5.6968	5.6855	0.0113	5.7288	-0.032	5.707	-0.0102
9	9	5.8508	5.7803	0.0705	5.7255	0.1253	5.8403	0.0105
10	10*	5.8239	5.831	-0.0071	5.8031	0.0208	5.6803	0.1436
11	11	5.6882	5.6486	0.0396	5.7569	-0.0687	5.7262	-0.038
12	12	5.5317	5.4941	0.0376	5.4936	0.0381	5.5148	0.0169
13	13	5.5317	5.5185	0.0132	5.5103	0.0214	5.5049	0.0268
14	14	5.7235	5.7718	-0.0483	5.7259	-0.0024	5.7287	-0.052
15	15	5.6003	5.5948	0.0055	5.5887	0.0116	5.5826	0.0177
16	16	5.7212	5.7392	-0.018	5.7225	-0.0013	5.7007	0.0205
17	17	5.7011	5.7306	-0.0295	5.7096	-0.0085	5.6926	0.0085
18	18	5.5952	5.5579	0.0373	5.5993	-0.0041	5.6122	-0.017
19	19	5.8356	5.8395	-0.0039	5.7667	0.0689	5.8518	-0.016
20	20	5.8356	5.8693	-0.0337	5.8214	0.0142	5.8163	0.0193
21	21*	6.3098	6.033	0.2768	5.9569	0.3529	5.7767	0.5331
22	22	5.6038	5.6064	-0.0026	5.6073	-0.0035	5.59	0.0138
23	23*	5.6038	5.607	-0.0032	5.5729	0.0309	5.674	-0.0702
24	24	5.8182	5.8841	-0.0659	5.8133	0.0049	5.8248	-0.006
25	25*	5.6861	5.69	-0.0039	5.7071	-0.021	5.6788	0.0073
26	26	5.8386	5.8515	-0.0129	5.814	0.0246	5.8172	0.0214
27	27	5.8125	5.8428	-0.0303	5.7902	0.0223	5.812	0.0005
28	28	5.6799	5.6702	0.0097	5.72	-0.0401	5.6794	0.0005
29	29	5.5272	5.4722	0.055	5.4996	0.0276	5.5121	0.0151
30	30	5.5272	5.4966	0.0306	5.5538	-0.0266	5.5431	-0.015
31	31	5.699	5.7499	-0.0509	5.7342	-0.0352	5.7438	-0.044
32	32	5.5952	5.5729	0.0223	5.5551	0.0401	5.6001	-0.004
33	33	5.7144	5.7172	-0.0028	5.7042	0.0102	5.7075	0.0069
34	34	5.6946	5.7086	-0.014	5.6796	0.015	5.6843	0.0103
35	35*	5.5272	5.576	-0.0488	5.6259	-0.0987	5.6851	-0.1579
36	36*	5.4535	5.485	-0.0315	5.5866	-0.1331	5.6115	-0.158
37	37	5.6968	5.6797	0.0171	5.6718	0.025	5.7013	-0.004
38	38*	5.5884	5.8	-0.2116	5.5441	0.0443	5.6629	-0.0745

39	39	5.4295	5.4469	-0.0174	5.4667	-0.0372	5.4294	0.0001
40	40*	5.4214	5.41	0.0114	5.4504	-0.029	5.6658	-0.2444

(*Test compounds)

Experiment

CoMFA and CoMSIA statistical results

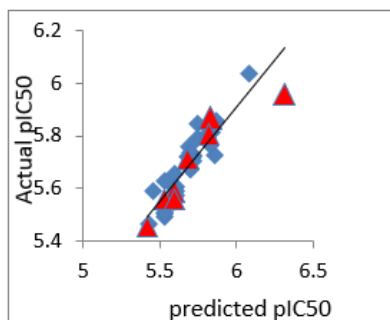
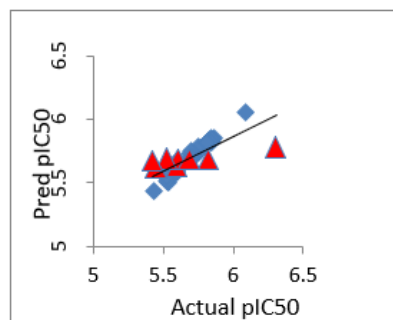
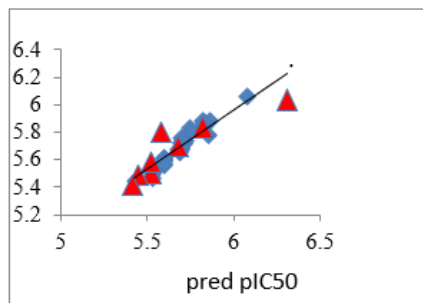
Different parameters such as correlation coefficient (r^2), cross-validated correlation coefficient (q^2) and standard error of estimate were taken in consideration while building the qsar model. Leave one out (LOO) cross validation was performed to find out cross validated r^2 (q^2) and number of component. After the cross validation run non cross validation run was perform to find out other parameters. Best generated CoMFA and CoMSIA models in Pullman charges having q^2 values of 0.783 and 0.892 respectively. The r^2 value of CoMFA and COMSIA analysis were 0.834 and 0.879 respectively. CoMFA steric field contribution was 0.899 while electrostatic field contribution was 0.401, which indicates

the dominant role of electron donating and electron withdrawing group in activity. CoMSIA analysis gives better statistical results in terms of cross validated r^2 (q^2). The CoMSIA analysis explores more fields as compare to CoMFA analysis. CoMSIA analysis reveals steric, electrostatic contributions as well as hydrophobic, and donor contributions which were listed in Table 2. The contributions of steric, electrostatic, donor, Acceptor and hydrophobic fields of best CoMSIA model were 0.141, 0.390, 0.207, 0.444 and 0.119 respectively.

The correlation graph between experimental pIC_{50} and predicted pIC_{50} for different CoMFA, CoMSIA and HQSAR model was shown in Figure 7. The predicted and residual values of models were shown in Table 2.

Table.2. The PLS statistics of CoMFA and CoMSIA models for Anti-malarial activity indicating the best model and field contribution.

Statistical parameters	CoMFA	CoMSIA	Field contribution	CoMFA	CoMSIA
q^2 (Cross validated)	0.783	0.892	Steric	0.899	0.141
r^2	0.834	0.879	Electrostatic	0.401	0.390
F value	51.903	91.279	Donor	-	0.207
SEE	0.021	0.040	Acceptor	-	0.444
Number of component	6	7	Hydrophobic	-	0.119
Pred R^2	0.882	0.788			

Fig.2 CoMFA graph between actual and predicted pIC₅₀Fig.3 CoMSIA graph between actual and predicted pIC₅₀Fig.4 HQSAR graph between actual and predicted pIC₅₀

Test Set



Training Set

CoMFA and CoMSIA contour map analysis

The PLS CoMFA contours maps in terms of common steric and electrostatic potential were generated taking compound 12 having greatest anti-malarial activity of the dataset suggesting that CoMFA steric interaction is favoured by steric/bulky groups in phenyl ring and CF₃ group attached to it at the 4th position at R₁, 5th position near CN group at phenyl ring and at the 1st position phenyl ring at R₂ (Green), while steric burden at ring near R₃ position 3' (Yellow) are supposed not to be favourable. CoMFA electrostatic interaction is favoured by electrostatic substitution at phenyl ring at 4th

position of CN group, 5th & 6th position of phenyl ring B and near the R₃ substitution ring (Blue), while electrostatic substitution at 4th position of CF₃ and above the benzimidazole ring at R₃ position (Red) are expected to be unfavourable (Figure 5)

Pose analysis of compound 12 with CoMFA steric parameter are suggested that phenyl ring and CF₃ group attached to it at the 4th position at R₁, 5th position near CN group in phenyl ring and at the 1st position phenyl ring at R₂ is not favouring the bulky groups addition, while R₃ is supported by addition of bulky groups.

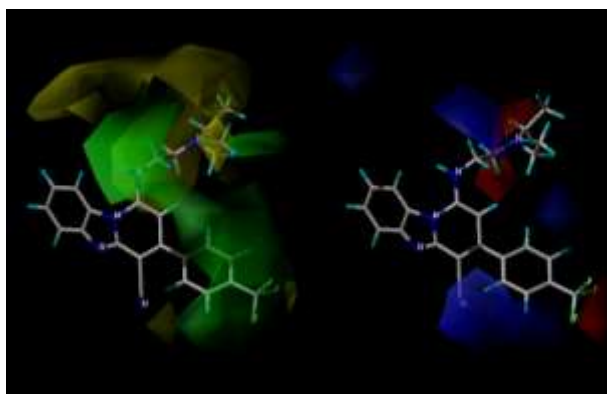
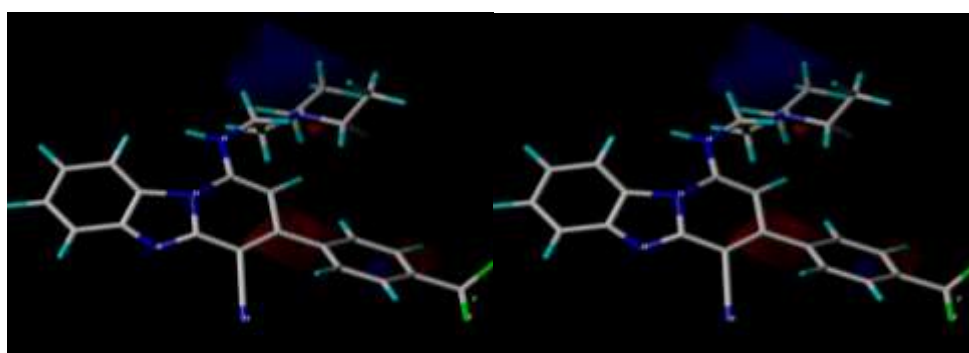


Fig.5 CoMFA contour Maps of compound 12 Steric(a) and Electrostatic

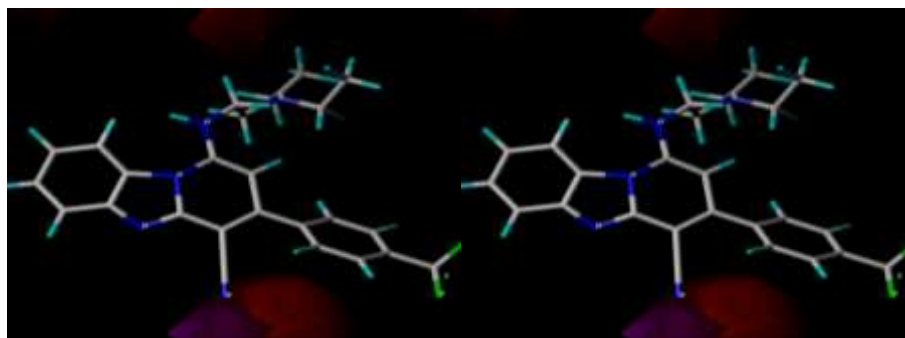
The PLS contours from CoMSIA analysis were carried out, taking the most active compound number 12. Steric interaction showed that phenyl ring at 1st position of R₂ is supposed to be not favouring (Yellow) while near phenyl ring at R₁ at 3, 4, 5 position and above the benzimidazole ring at the R₃ position of the ethyl ring (Green) steric group. Addition of small non bulky group at phenyl ring at 1st position of R₂ is supposed to enhance anti-malarial activity while at a near phenyl ring at R₁ at 3, 4, 5 position and above the benzimidazole ring at the R₃ position, enhance the anti-malarial activity. Electrophilic interaction showed that above the

benzimidazole ring at the R₃ position strongly favoured by electron donor (Blue) and while the phenyl ring at R₁ shows withdrawal group (Red). Hydrophobic phenyl ring at 1st position, 3rd position of CF₃ and the benzimidazole ring at the R₃ position not support the hydrophobic substitution (White). Hydrophobic substitution is also supposed to be not favouring at the position R₃ above the benzimidazole ring. Donor group supposed to support activity at position 8th benzimidazole and interaction showed that above the benzimidazole ring at the R₃ position (Magenta) and acceptor group at 4th of the CN position of a ring.



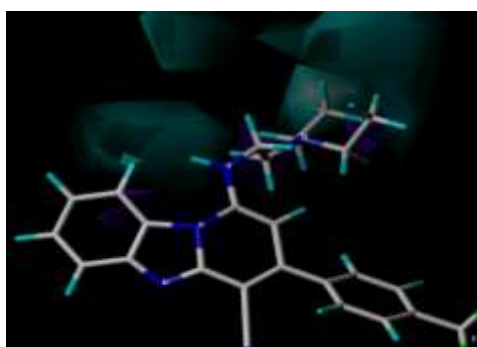
(a) Steric

(b) Electrostatic



(c)Hydrophobic

(d) Donor



(e) Acceptor

HQSAR studies

The best HQSAR model for Anti-malarial was generated using Atom (A) bonds (B) Donor (D)

Acceptor (A) as fragment distinction parameters and 2-5 as the fragment size, showing $q^2 = 0.935$ and $r^2 = 0.971$ at 6 optimum numbers of components and 199 hologram lengths was selected. For Anti-malarial, the another best HQSAR model was developed using atoms (A) bonds (B) Chirality Hydrogen (Ch) Donar Acceptor (DA) as fragment distinction parameters and 2-5 as the fragment size,

showing $q^2 = 0.935$ and $r^2 = 0.971$ at 6 optimum numbers of components and 151 hologram lengths was selected. It is interesting to note that the both best model of data set was generated when long fragments (i.e., 6-10 for anti-malarial activity) were not considered. Notably, there is a inverse relationship between the side chain length and the potency.

Table.3. Summary of statistical parameters of HQSAR study

S. No.	Statistical parameters	Fragment distinctions	
		Model 1(A/B/DA)	Model 2(A/B/Ch/DA)
1.	Fragment size	2-5	2-5
2.	r^2 best cv	0.971	0.971
3.	q^2 best full	0.935	0.935
4.	r^2 Ensemble	0.967	0.967
5.	Standard error	0.025	0.025
6.	Number of component	6	6
7.	Best hologram length(bin)	199	151

HQSAR contour maps analyses:

In HQSAR contour map, red and orange colour shows negative contribution while green and yellow colour shows positive contribution to activity. In the HQSAR contour of compound number 12, a orange and red contour is seen in the phenyl ring which shows the phenyl ring 4,5

position near CN group enhance the anti malarial activity and yellow colour near R₃ position above the imidazole ring positive contribution to activity.

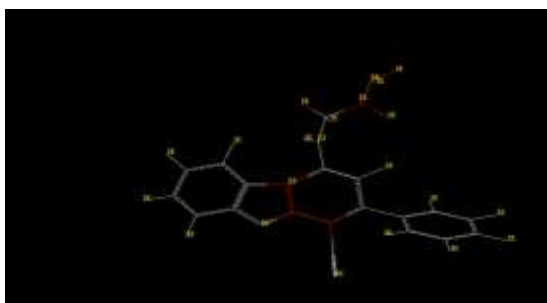


Fig. 6 HQSAR Molecular fragment pose compound 12. Orange and red fragment showing favourable part for activity.

Docking studies

Molecular docking studies was carried out using the Surflex Dock module in SYBYL X2.0 software. The protein structure of pdb name along with their inhibitor was retrieved from RCSB Protein Data Bank (PDB entry code: 2ANL). The protein structures were subjected

to energy minimization and charge calculation (AMBER7FF99). The bloat values was set as 1 and the threshold values as 0.5 for generation of protomol and position was considered to be the active sites for potential receptor's binding sites.

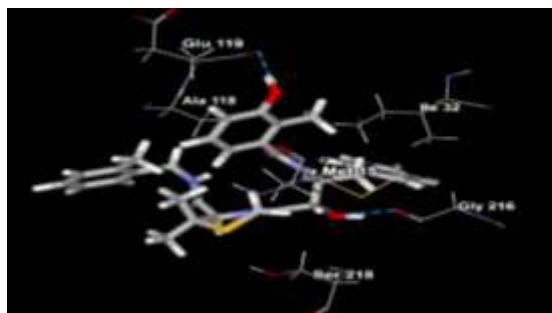


Fig.7 Docking pose of most active compound

Designing of novel benzimidazoles

On the basis of reported structure activity relationship of pyrido[1,2-a] benzimidazole analogues as an antimalarial QSAR studies

using CoMFA, CoMSIA, HQSAR, and Molecular modelling (Docking) studies twenty compounds were designed.

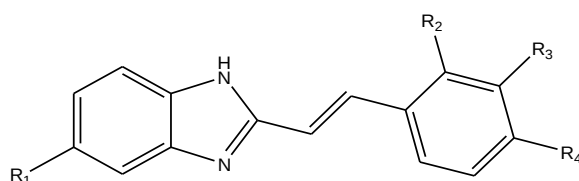


Table.4. Designed benzimidazole analogues on the basis of computational studies with their predicted

Compounds id	CoMFA	CoMSIA	HQSAR	MolDock Score
SY- 01	4.5845	4.5796	4.85798	-100.116
SY- 02	4.6871	4.5871	4.5799	-107.516
SY- 03	5.2147	5.3214	5.6987	-100.176
SY- 04	5.3489	5.3217	5.2147	-99.945
SY- 05	5.3698	5.6874	5.3214	-100.328
SY- 06	5.8741	5.2879	6.2469	-98.6436
SY- 07	5.6987	5.3297	5.2879	-98.180
SY- 08	5.2497	5.1264	5.3298	-91.529
SY- 09	6.2587	4.5478	6.2547	-117.34
SY- 10	5.2587	5.2558	5.5587	-90.41
SY- 11	5.2469	5.2167	5.2689	-112.59
SY- 12	5.2149	5.0240	5.0001	-105.179
SY- 13	5.3250	5.0219	5.0349	-106.653
SY- 14	5.6934	5.2149	5.1527	-103.888
SY- 15	5.2413	5.3241	5.2143	-115.047
SY- 16	5.2164	5.2143	5.2149	-104.192
SY- 17	5.0120	5.2010	5.0240	-115.434
SY- 18	5.2401	5.2497	5.2149	-112.272
SY- 19	5.2469	5.23	5.3497	-112.604
SY- 20	5.3697	5.2147	5.8797	-105.203

Synthesis of designed compounds

A procedure was employed to synthesize the designed compounds. Substituted ortho-phenylenediamine (0.01mol) weight 1.081gm

and trans-cinnamic acid (0.01 mol) weight 1.481gm solution was prepared in ethylene glycol (10 ml) in a 100mL round bottom flask. The reaction mixture was subjected to heated at

200°C for 6-18 hours on oil bath. The reaction mixture was poured into ice water and filtered, washed with water and extract with ethyl acetate and water with the help of separating funnel by phase separation then the product was passed from anhydrous sodium sulphate from funnel and the product was recrystallized using ethanol. [9]

SY- 01: 2-styryl-1H-benzo [d] imidazole

Yield 78.52 %, m.p.: 155-158 °C, Rf value-0.62, IR spectrum, ν , cm^{-1} : 1627 (Ar), 3286, 3109 (N-H, Str), 1627, 1542 (N-H, Bend), 1697, 1627 (C=N), 1697, 1542 (C=C), 1627 & 1442 (Ar), ^1H NMR spectrum (CDCl_3), δ , ppm : 7.26; 7.28; 7.71; 7.75 (q, 4H, Ar - H), 4.49 (s, 1H, N - H), 6.92; 6.94 (d, 1H, but - CH), 7.15; 7.21; 7.30 (m, 5H, Ar - H), ^{13}C NMR spectrum (CDCl_3), δ , ppm : 115.4; 122.4; 123.4; 115.4; 137.86; 139.1 (m, 6C, Ar - C), 142.35 (s, 1C, N- C), 113.2; 134.1 (d, 2C, but- CH), 127.06; 128.17; 128.17; 128.16; 127.06; 135.3 (m, 6C, Ar - C). LCMS Mass spectrum, m/z: 220 [M^+].

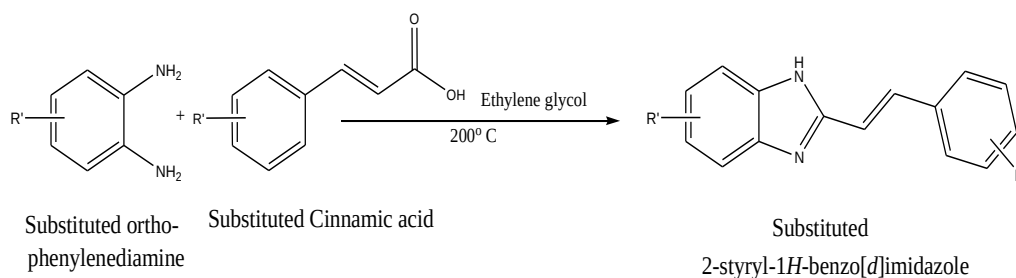
SY- 02: 2-(4-nitrostyryl)-1H benzoimidazole

Yield 58.58 %, m.p.: 135-137 °C, Rf value-

0.69, IR spectrum, ν , cm^{-1} : 1596 & 1350 (Ar), 3471, 2985 (N-H, Str), 1666, 1573 (N-H, Bend), 1704, 1666 (C=N), 1704, 1596 (C=C), 1573 & 1350 (NO_2), ^1H NMR spectrum (CDCl_3), δ , ppm : 7.66; 7.26; 7.26; 7.66 (q, 4H, Ar - H), 4.39 (s, 1H, N - H), 7.13; 7.28 (d, 1H, but - CH), 7.56; 7.57; 8.14; 8.14 (q, 4H, Ar - H), ^{13}C NMR spectrum (CDCl_3), δ , ppm : 114.92; 140.27; 141.92; 114.92; 123.4; 123.9 (m, 6C, Ar - C), 141.9 (s, 1C, N- C), 114.9; 132.51 (d, 2C, but- CH), 127.3; 121.8; 147.19; 120.8; 127.03; 141.9 (m, 6C, Ar - C). LCMS Mass spectrum, m/z: 263 [M^+ 2].

SY- 03: 2-(3-Nitrostyryl)-1H- benzoimidazole

Yield 32.62 %, m.p.: 117-120 °C, Rf value-0.63, IR spectrum, ν , cm^{-1} : 1596 & 1350 (Ar), 3471, 2985 (N-H, Str), 1666, 1573 (N-H, Bend), 1704, 1666 (C=N), 1704, 1596 (C=C), 1573 & 1350 (NO_2), ^1H NMR spectrum (CDCl_3), δ , ppm : 7.70; 7.26; 7.26; 7.70 (q, 4H, Ar - H), 5.0 (s, 1H, N - H), 7.24; 7.10 (d, 1H, but - CH), 7.69; 7.47; 8.07; 8.23 (q, 4H, Ar - H), LCMS Mass spectrum, m/z: 265.



Synthetic scheme

SY- 04: 5-Nitro-2-styryl-1H- benzoimidazole

Yield 65.53 %, m.p.: 112-115 °C, Rf value-0.52, IR spectrum, ν , cm^{-1} : 1596 & 1350 (Ar), 3471, 2985 (N-H, Str), 1666, 1573 (N-H, Bend), 1704, 1666 (C=N), 1704, 1596 (C=C), 1573&1350 (NO_2), ^1H NMR spectrum (CDCl_3), δ , ppm : 7.96; 8.19; 8.63 (t, 4H, Ar - H), 5.49 (s, 1H, N - H), 6.99; 6.99 (d, 1H, but - CH), 7.90; 7.21; 7.14; 7.21 (m, 5H, Ar - H), LCMS Mass spectrum, m/z: 266.

SY- 07: 2-(3,4-dimethoxyphenyl)-1H- benzoimidazole

Yield 76.32 %, m.p.: 181-184 °C, Rf value-0.52, IR spectrum, ν , cm^{-1} : 1589 & 1357 (Ar), 3456, 3116 (N-H, Str), 1666, 1558

(N-H, Bend), 1712, 1656 (C=N), 1586 & 1359 (Ar), 1558 & 1357 (CH_3), ^1H NMR spectrum (CDCl_3), δ , ppm : 7.70; 7.26; 7.26; 7.70 (q, 3H, Ar - H), 5.08 (s, 1H, N - H), 6.88; 6.93; 6.72 (t, 4H, Ar - H), 3.73; 3.73 (d, 1H, OCH_3), LCMS Mass spectrum, m/z: 254.

SY- 08: 2-(3-nitrostyryl)-5-nitro-1H- benzoimidazole

Yield 67.88 %, m.p.: 150-153 °C, Rf value-0.64, IR spectrum, ν , cm^{-1} : 1542 & 1450 (Ar), 1542 & 1342 (NO_2), 3496, 3062 (N-H, Str), 1643, 1542 (N-H, Bend), 1728, 1643 (C=N), 1643, 1542 (C=C), ^1H NMR spectrum (CDCl_3), δ , ppm : 7.90; 8.19; 8.47 (t, 3H, Ar - H), 4.22 (s, 1H, N - H), 7.41; 7.42 (d, 2H, but - CH), 7.46; 7.68; 8.10; 8.23 (q, 4H, Ar - H), LCMS Mass spectrum, m/z: 310.

SY- 09: 2-(4-methoxystyryl)-1H benzimidazole Yield 20.27 %, m.p.: 155-158 °C, Rf value-0.61, IR spectrum, ν , cm^{-1} : 1627 & 1442 (Ar), 3394, 2977 (N-H, Str), 1627, 1496 (N-H, Bend), 1735, 1627 (C=N), 1735, 1591 (C=C), 1442 & 1375 (CH_3)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.79; 7.79; 8.19; 8.43 (q, 4H, Ar - H), 5.22 (s, 1H, N - H), 6.84; 6.98 (d, 2H, but - CH), 7.17; 7.23; 6.60; 6.70 (q, 4H, Ar - H), 4.12 (s, 1H, OCH_3)., LCMS Mass spectrum, m/z: 250.

SY- 10: 2-(3, 4-dimethoxyphenyl)-5-nitro-1H-indene Yield 51.11 %, m.p.: 117-120 °C, Rf value-0.81, IR spectrum, ν , cm^{-1} : 1596 & 1249 (NO_2), 1598 & 1400 (Ar), 3417, 3116 (N-H, Str), 1620, 1560 (N-H, Bend), 1712, 1620 (C=N), 1596 & 1435 (Ar), 1596 & 1249 (CH_3)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.96; 8.19; 8.63 (t, 3H, Ar - H), 5.0 (s, 1H, N - H), 6.98; 6.93; 6.77 (t, 4H, Ar - H), 3.79; 3.73 (d, 1H, OCH_3)., LCMS Mass spectrum, m/z: 299.

SY- 11: 2-(4-methoxystyryl)-5-nitro-1H benzoimidazole Yield 54.42 %, m.p.: 119-122 °C, Rf value-0.79, IR spectrum, ν , cm^{-1} : 1612 & 1508 (Ar), 1573 & 1380 (NO_2), 3379, 3116 (N-H, Str), 1612, 1573 (N-H, Bend), 1666, 1612 (C=N), 1712, 1612 (C=C), 1504 & 1380 (CH_3 , Bend)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.79; 8.19; 8.43 (t, 3H, Ar - H), 5.08 (s, 1H, N - H), 6.99; 6.98 (d, 2H, but - CH), 7.17; 7.19; 6.76; 6.77 (q, 4H, Ar - H), 3.73 (s, 1H, OCH_3)., LCMS Mass spectrum, m/z: 297 [M^+2].

SY- 12: 2-(4-nitrophenyl)-1H-benzoimidazole Yield 38.32 %, m.p.: 144-147 °C, Rf value-0.79, IR spectrum, ν , cm^{-1} : 1573 & 1512 (Ar), 3479, 3101 (N-H, Str), 1666, 1550 (N-H, Bend), 1743, 1660 (C=N), 1572 & 1512 (Ar), 1550 & 1330 (NO_2)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.70; 7.26; 7.26; 7.70 (q, 4H, Ar - H), 5.0 (s, 1H, N - H), 7.74; 8.23; 8.20; 7.74 (q, 4H, Ar - H)., LCMS Mass spectrum, m/z: 239.

SY- 14: 5-nitro-2-(3-nitrophenyl)-1H-benzoimidazole Yield 70.28 %, m.p.: 201-204 °C, Rf value-0.64, IR spectrum, ν , cm^{-1} : 1473 & 1373 (NO_2), 1612 & 1473 (Ar), 3479, 3093 (N-H, Str), 1635, 1589 (N-H, Bend), 1635, 1612 (C=N), 1589 & 1473 (Ar), 1479 & 1373 (NO_2)., ^1H NMR spectrum (CDCl_3), δ , ppm :

7.96 8.19; 8.63 (t, 4H, Ar - H), 5.0 (s, 1H, N - H), 8.47; 8.13; 7.87; 7.58 (q, 4H, Ar - H)., LCMS Mass spectrum, m/z: 284.

SY- 15: 2-(4-nitrostyryl)-5-nitro-1H benzoimidazole Yield 79.95 %, m.p.: 194-197 °C, Rf value-0.52, IR spectrum, ν , cm^{-1} : 1589 & 1473 (Ar), 1550 & 1473 (NO_2), 3471, 3070 (N-H, Str), 1643, 1550 (N-H, Bend), 1681, 1643 (C=N), 1681, 1589 (C=C), 1589 (Ar), 1550 (NO_2)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.94; 8.20; 8.49 (t, 3H, Ar - H), 5.95 (s, 1H, N - H), 7.40; 7.41 (d, 1H, but - CH), 7.67; 7.69; 8.14; 8.14 (q, 4H, Ar - H)., ^{13}C NMR spectrum (CDCl_3), δ , ppm : 115.4; 143.13; 136.3; 108.24; 142.7; 115.41 (m, 6C, Ar - C), 141.72 (s, 1C, N - C), 111.37; 133.76 (d, 2C, but - CH), 120.80; 147.89; 120.08; 127.90; 141.67; 128.10 (m, 6C, Ar - C). LCMS Mass spectrum, m/z: 310.

SY- 16: 2-(2-nitrostyryl)-5-nitro-1H benzoimidazole Yield 69.98 %, m.p.: 178-181 °C, Rf value-0.51, IR spectrum, ν , cm^{-1} : 1589 & 1442 (Ar), 1558 & 1380 (NO_2), 3479, 3209 (N-H, Str), 1589, 1558 (N-H, Bend), 1712, 1589 (C=N), 1712, 1589 (C=C), 1589 & 1442 (Ar), 1550 & 1380 (NO_2)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.97; 8.15; 8.06 (t, 3H, Ar - H), 5.88 (s, 1H, N - H), 7.43; 7.28 (d, 2H, but - CH), 7.42; 7.61; 7.40; 8.15 (q, 4H, Ar - H)., LCMS Mass spectrum, m/z: 310.

SY- 18: 2-(2,4-dichlorostyryl)-5-nitro-1H benzoimidazole Yield 80.23 %, m.p.: 180-183 °C, Rf value-0.57, IR spectrum, ν , cm^{-1} : 1596 & 1450 (Ar), 1558 & 1334 (NO_2), 3394, 3085 (N-H, Str), 1650, 1558 (N-H, Bend), 1720, 1650 (C=N), 1690, 1596 (C=C), 1650 & 1450 (Ar), 786, 555 (Cl)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.91; 8.18; 7.89 (t, 3H, Ar - H), 5.91 (s, 1H, N - H), 6.68; 6.64 (d, 2H, but - CH), 7.38; 7.38; 7.39; 7.40 (q, 4H, Ar - H)., LCMS Mass spectrum, m/z: 337 [M^+3].

SY- 20: 2-(4-chlorostyryl)-5-nitro-1H benzoimidazole Yield 32.21 %, m.p.: 115-117 °C, Rf value-0.53, IR spectrum, ν , cm^{-1} : 1497 & 1399 (Ar), 3294, 3185 (N-H, Str), 1598, 1600 (N-H, Bend), 1728, 1640 (C=N), 1640, 1586 (C=C), 1500 & 1497 (Ar), 786, 555 (Cl)., ^1H NMR spectrum (CDCl_3), δ , ppm : 7.71; 7.24; 7.64 (t, 3H, Ar - H), 4.06 (s, 1H, N - H), 6.78; 6.84 (d, 2H, but - CH), 7.38; 7.27; 7.03; 7.24 (

q, 4H, Ar - H), LCMS Mass spectrum, m/z: 288 [M⁺].

Results and discussion

Chemistry

The synthesis of Styrl benzimidazole derivatives were commenced with the synthesis of Substituted ortho-phenylenediamine and substituted cinnamic acid solution was prepared in ethylene glycol. The reaction mixture was subjected to heat at 200°C for six-fourteen hours on oil bath. The reaction mixture was poured into ice water and filtered, washed with water and extract with ethyl acetate and water with the help of separating funnel by phase separation then the product was passed from anhydrous sodium sulphate from funnel and the product was recrystallized using ethanol. The product was dried and used for further analysis. [9]

In Vitro Antimalarial Screening

All the synthesized compounds were screened for anti malarial activity in the Microcare laboratory & TRC, Surat, Gujarat.

The in vitro anti malarial assay was carried out in 96 well microtitre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. [10] The cultures of *P.falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage

parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O+). [11] A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. [12,13] The culture plates were incubated at 37 °C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasite into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine was used as the reference drug.[14]

Observations of the in Vitro Antimalarial Screening

The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percent maturation inhibition with respect to control group. The minimum inhibitory concentration (MIC) values (in the Table 5).

Table.5. In-vitro Anti-malarial activity of the synthesized compounds

S. No	Compound ID	IC ₅₀ (µg/ml)
01	SY-01	0.96
02	SY-02	0.85
03	SY-03	1.14
04	SY-04	1.02
05	SY-07	0.96
06	SY-08	1.35
07	SY-09	0.84
08	SY-11	1.07
09	SY-12	0.97
10	SY-14	1.30

11	SY-15	1.10
12	SY-16	0.94
13	SY-17	1.56
14	SY-18	0.94
15	SY-20	0.86
16	Chloroquine	0.020
17	Quinine	0.268

In-vitro Anti-malarial activity of the synthesized compounds

Many of the compounds showed anti malarial activity comparable to that of chloroquine and Quinine (IC_{50} = 0.84 -0.97 μ g/ml), with compound SY-09 being the most active (IC_{50} =0.84 μ g/ml less potent than chloroquine). The compound SY-09 shows an active potent as compared to the other synthesised compounds. The compound shows good effect on *P. falciparum* strain because of the substitution of the OCH_3 group at R^4 position which is electron donating group while the compound SY-11 does not shows good activity because the substitution of NO_2 group at the R^4 position on the (A) phenyl ring as we know that NO_2 is electron withdrawing group in nature and the compound SY-20 is less active compound as compared to the SY-09 the compound SY-20 contains substitution of chloro group at (A&C) phenyl ring R^4 position the Cl group is electron withdrawing in nature and the compound SY-18 does not show good activity as compared to SY-20 the compound SY-18 contains the substitution of the NO_2 group at R^4 position at (A) phenyl ring and two Cl group at R^2 & R^4 position at (C) the nitro group is electron withdrawing group in nature which decrease the anti malarial activity of the compound. The compound SY-02 shows good inhibitory result as compared to the SY-01 the SY-02 contains substitution of nitro group at R^4 position at (C) ring but SY-01 does not contain any substitution so it is less active than SY-02.

The compound SY-17 shows less potent inhibitory as compared to the other synthesised compounds because of the substitution of the nitro group at R^4 position of (A) phenyl ring and substitution of OCH_3 group at R^3 & R^4 position at (C) ring the compound SY-08 also shows less inhibitory result as compared to the compound SY-17. The compound SY-08 consist of the

substitution of nitro group at R^4 position at (A) phenyl ring and R^3 position at (C) ring as we know that the nitro group is good electron withdrawing group in nature which decrease the anti malarial activity of the compound.

Conclusion

This study describes QSAR and docking studies for designing of novel anti-malarial. Three QSAR methods, CoMFA, CoMSIA and HQSAR were used to investigate the relationship between the structures of 40 compounds and activities in order to get new clues to develop new potent anti-malarial. The CoMFA, CoMSIA and HQSAR models showed meaningful statistical significant results in terms of internal validation (q^2) of Pyrido[1,2]a benzimidazoles derivatives. The high q^2 obtained from these different QSAR methods suggest that we successfully got three rational and predictive QSAR models. The explored CoMFA and CoMSIA models provided information about favorable and unfavorable region while HQSAR provides information about positive, negative and intermediate contribution of sub-structural fingerprint requirements for imparting the biological activity. The CoMFA, CoMSIA and HQSAR contour maps revealed sufficient information to understand the structure activity relationship (SAR) and to recognize structural features influencing inhibitory activity. The binding mode of the Pyrido[1,2]a benzimidazoles analogues was clarified by the flexible docking method and hydrogen bonding interactions were found to be important for the binding on PDB 2ANL. Based on the SAR and docking studies generated by molecular modelling analysis, 15 anti-malarial were successfully designed exhibiting good predicted activities in all applied computational approach. All this information proved helpful in designing of

Styryl-benzimidazoles derivatives as potent anti-malarial agents.

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