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Thiosemicarbazone and Benzimidazole Hybrid Molecules: The Privileged Scaffolds for Anti-Malarial Activity

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Abstract

Plasmodium falciparum Enoyl-ACP Reductase is an important target for antimalarial drugs. Despite the development of resistance against PfENR inhibitors drugs, there is still significant potential for designing new chemical entity with affordable, safe and efficacious antimalarials.In present study thiosemicarbazone-benzimidazole hybrids were designed and interaction of these conjugate hybrids was investigated by docking studies in the binding site of PfENR (PDB ID:3AM5) enzyme using Glide v 5.6. Among the series of designed compounds seven compounds with good potential were synthesized. Structural confirmation of these compounds was done by FT-IR, 1H-NMR and Mass spectroscopy. The compounds were evaluated for *in vitro* antimalarial activity against resistant strain of *plasmodium falciparum* by microdilution technique. The activity of compound AD13 was found to be comparable with chloroquine and better than the quinine. The above study could be very useful for further design and development of new antimalarials.

Keywords: *PfENR , thiosemicarbazone-benzimidazole hybrids, docking, antimalarials.*

1. Introduction

Malaria is a life-threatening disease caused by Plasmodium parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes, called "malaria vectors." According to the latest WHO estimates, released in December 2016, there were 214 million cases of malaria in 2016 and 438 000 deaths. Between 2000 and 2015, malaria incidence among populations at risk fell by 37% globally; during the same period, malaria mortality rates among populations at risk decreased by 60%[1][2].

Despite continuous research efforts, malaria continuous to exert the tremendous burden on the health due to development of resistance to currently available antimalarial like Chloroquine and 4-aminoquinolines. Therefore, there is urgent need to develop new affordable, safe, and efficacious antimalarials. Among the available ways to optimize the research for new therapeutic agents, various techniques are being adopted: for example, the use of hybrid compounds is considered an extension of the concept of combination therapy, where the coupling of two pharmacophoric groups, often covalently joined, is observed, creating a single chemical entity capable of modulating multiple targets. The use of hybrid drugs is an interesting way to discover new drugs, making it possible to circumvent the resistance of parasites, a phenomenon that appears to be composition-specific and not related to the changes in the action of the drug target. With these thoughts, various research groups have synthesized a large number of hybrid molecules by combination of chloroquine with different pharmacophores acting on different targets. The most common antimalarial agents from these studies include hybrid based on chloroquine and thiazolidinone scaffolds[4], keto-enamine chalcone-chloroquine hybrids[5], 4-aminoquinoline-pyrimidine hybrids[6][11]. Some of these hybrids have shown promising *in-vitro* and *in-vivo* antimalarial activity against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum.*

To overcome the resistance piperaquine, hydroxypiperaquine and dichloroquinazine exhibited promising antimalarial efficacy but toxic liabilities ruled out their development as drug candidate. Benzimidazole nucleus and purine base of the DNA are having same structure. When Benzimidazole combined with thiosemicarbazone and due to its iron-chelating properties overcome rapid development of anti-malarial resistance problems[3]. Encouraged by these results and research towards the synthesis of novel antimalarial agents designed a new series of thiosemicarbazone-benzimidazole hybrids to develop structurally diverse series of compounds in order to gain structural insight for improved antimalarial activity. Thus in the present study, we report the synthesis and antimalarial activity of a new series of thiosemicarbazone-benzimidazole hybrids.

2. Materials and methods

2.1 Computational studies

2.1.1 Molecular Docking

Docking studies were carried out using Glide v5.6, Schrödinger LLC, NewYork (http:// www. Schrodinger.com). The computational process of searching
for a ligand that is able to fit both geometrically and energetically into the binding site of protein is called molecular docking. All the compounds were docked in active site of protein [PDB ID: 3AM5]. Molecular docking studies involved ligand preparation, protein preparation, receptor grid generation, docking studies and further analysis of docking results. The steps involved in docking studies are as follows:

2.1.2 Chem Draw Ultra 2D 8.0

The 2Dstructure of thiosemicarbazone and benzimidazole hybrid derivative was drawn by using Chem Draw Ultra 8.0 developed by Cambridge Pvt. Ltd and the structure of each

compound was analyzed for correction error in bond order and converted to 3D structure with the help of 3D optimization tool was saved in .mol file compatible with maestro format.

2.1.3 Ligand preparation

All the built compound structures with their 3D .mol file were imported in maestro v9.1. By using the LigPrep version 2.4 (2010), the drawn ligand was geometry optimized and partial atomic charges were computed by using the Optimized Potentials for Liquid Simulations 2005 (OPLS 2005) force field. Various possible ionization states were generated at pH 7 ± 2.0 to generate single low energy 3-D structures for each of the input structure and the rest of the parameter values by defaults[7].

2.1.4 Protein preparation

The three dimensional structure of [PDB ID:3AM5] was obtained from the RCSB protein data bank (http://www.rcsb.org/pdb), the best proteins were selected by analyzing the protein with Ramachandran plot and regions. After selection, Protein preparation wizard (2010) of Schrodinger suite has been used to prepare protein. The proteins were preprocessed, the bond orders were assigned to residues of proteins, hydrogen atoms were added and tautomeric states at their normal pH (7.0) were generated. Impref minimization was carried out using the OPLS 2005 molecular mechanics force field with cut off RMSD of 0.3 Å[8].

2.1.5 Receptor grid generation

Minimized protein was used for grid generation which involves selected ligand as the reference as it signifies the binding sites of drug with respect to the target. The active site is generally represented as an enclosing box at the centroid of workspace ligand. All ligands were docked into this grid structure[6].

2.1.7 Docking studies

Ligand docking was done by using Glide, v 5.6. The prepared ligands and the file obtained from receptor grid generation panel were selected and all the designed hybrids of thiosemicarbazone and benzimidazole derivatives were docked within the binding site of 3AM5. Flexible docking was done by employing Extra Precision (XP) mode of Glide. Glide score of compounds was obtained and various interaction of ligand with protein was studied. The final energy evaluation was done with the GlideScore and a single best pose was generated as output for a particular ligand with the help of following equation.

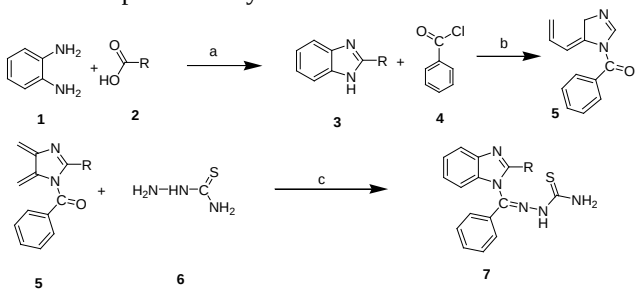
GScore = a*vdW+ b*Coul + Lipo + H bond + Metal + Bury P+ RotB + Site

Where vdW = Vander Waal energy, Coul = Coulomb energy, Lipo = Lipophilic contact term, HBond = Hydrogen-bonding term, Metal = Metal-binding term, BuryP = Penalty for buried polar group, RotB = Penalty for freezing rotable bonds, Site = Polar interaction at active site, and the coefficient of vdW and Coul are a = 0.065, b = 0.0130. The best pose for a given ligand was determined by the Emodel score, while different compounds were ranked using Glide score[6]

2.2 Synthesis

2.2.1 Chemistry

A series of twenty 2-substituted-1H-benzo[d]imidazol-1-yl(phenyl)methylene thiosemicarbazide AD2, AD7, AD8, AD10, AD13, AD19, AD20 derivatives were synthesized according to the synthetic route presented in Scheme 1. One of the key intermediates,2-substituted-1*H*-benzo[d]imidazole (3) was synthesized by reacting substituted aldehydes and acids with *o*-phenylenediamine in the presence of ammonium chloride and ethanol. The 2-substituted-1*H*-benzo[d]imidazole-1-yl) methanone(5) were synthesized by the reaction between 2-substituted-1*H*-benzo[d]imidazole (3) and benzoyl chloride(4) in the presence of sodium hydrogen carbonate and dilute HCl. 2-substituted-1H-benzo[d]imidazol-1-yl(phenyl)methylene thiosemicarbazide seven compound (AD2, AD7, AD8, AD10, AD13, AD19 and AD20) were synthesized by reacting with second intermediate 2-substituted-1*H*-benzo[d]imidazole-1-yl)methanone(5)& thiosemicarbazide in the presence of acetic acid and ethanol. The formation of compounds was confirmed by IR, 1H NMR and Mass spectral analysis.



Scheme 1-Synthetic scheme of Thiosemicarbazone derivatives.

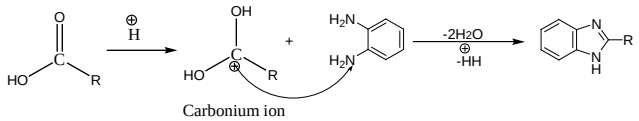
Reagents and conditions :-

1. NH_4Cl, ethanol, 80-90 ^oC, 2 hr stirring
2. $NaHCO_3$, dilute HCl
3. Ethanol, Acetic acid 70-80 ^oC, 4 hr reflux

2.2.2 Mechanism of Reaction

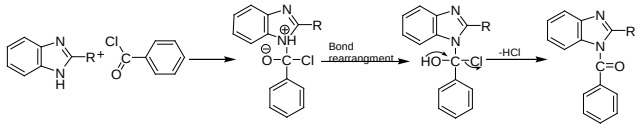
Step-1 Mechanism of synthesis of 2-substituted-1*H*-benzo[d]imidazole(3)

The role of ammonium chloride was to activate carboxyl group by addition of proton to carbonium ion. The reaction mechanism involved carbonium ion intermediate and eliminated two H_2O molecule.



Step-2 Mechanism of synthesis of 2-(substituted-1*H*-benzo[d]imidazole-1-yl)methanone(5)

Nucleophilic attack of nitrogen on electrophilic carbon of benzoyl chloride and eliminated HCl after bond rearrangement.



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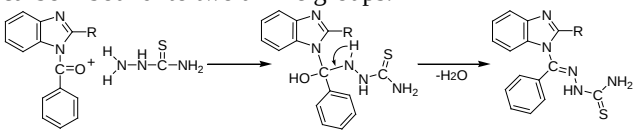
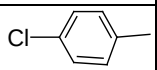
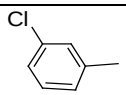
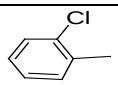
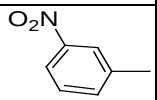
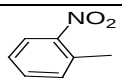
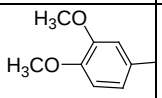
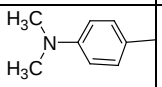
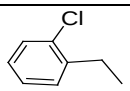
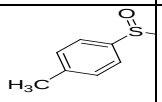
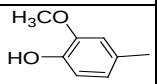
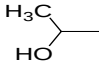
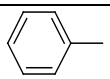
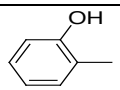
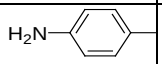
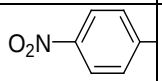
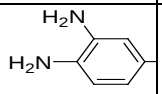
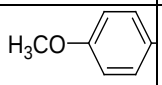
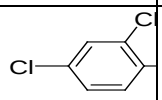
Step-3Mechanism of synthesis of 2-(substituted-1*H*-benzo[d]imidazole-1-yl)(Phenyl)(methylene) thiosemicarbazide (7)

Nucleophilic attack of Nitrogen on electrophilic carbonyl carbon.After that it formed hemiacetal and hemiketal.Than it is attacked by a second amine to form a compound with a carbon bound to two amine groups.



Nitrogen was deprotonated & electrons from this N-H bond push the oxygen off of the carbon leaving C=N double bond (an imine or Schiff base) & displaced water molecule.

TableNo.1-Compound code, substitutions & IUPAC name of Thiosemicarbazide- benzimidazole hybrids (AD1-AD20)

S. no.	Com. Code	R	IUPAC Name
1	AD1		(Z)-1-((2-(4-chlorophenyl)-1H-benzo[d] imidazol-1-yl) (phenyl)methylene) thiosemicarbazide
2	AD2		(Z)-1-((2-(3-chlorophenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
3	AD3		(E)-1-((2-(2-chlorophenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
4	AD4		(E)-1-((2-(3-nitrophenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
5	AD5		(E)-1-((2-(2-nitrophenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
6	AD6		(E)-1-((2-(3,4-dimethoxyphenyl)-1H-benzo [d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
7	AD7		(E)-1-((2-(4-(dimethylamino)phenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
8	AD8		(E)-1-((2-(2-chlorobenzyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
9	AD9		(E)-1-(phenyl(2-(p-tolylsulfinyl)-1H-benzo [d]imidazol-1-yl)methylene) thiosemicarbazide
10	AD10		(E)-1-((2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)(phenyl) methylene) thiosemicarbazide
11	AD11		(E)-1-((2-(1-hydroxyethyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene) thiosemicarbazide
12	AD12	-H	(E)-1-((1H-benzo[d]imidazol-1-yl) (phenyl) methylene)thiosemicarbazide
13	AD13	-CH3	(E)-1-((2-methyl-1H-benzo[d]imidazol-1 syl)(phenyl)methylene)thiosemicarbazide
14	AD14		(E)-1-(phenyl(2-phenyl-1H-benzo[d] imidazol-1-yl)methylene)thiosemicarbazide
15	AD15		(E)-1-((2-(2-hydroxyphenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
16	AD16		(E)-1-((2-(4-aminophenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
17	AD17		(E)-1-((2-(4-nitrophenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
18	AD18		(E)-1-((2-(3,4-diaminophenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
19	AD19		(E)-1-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide
20	AD20		(E)-1-((2-(2,4-dichlorophenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene)thiosemicarbazide

2.2.3 General procedure for synthesis of thiosemicarbazone and benzimidazole hybrids (AD2,AD7,AD8.AD10,AD13,AD19, and AD20)

A mixture of *o*-phenylenediamine (0.92mmol) and substituted aldehydes and acids (0.92mmol) in 4 ml of ethanol was added NH_4Cl (30 mol%). The resulting mixture was stirred for 2 hr at 80^oC. The completion of the reaction was confirmed by TLC ethylacetate:hexane, (2:1 v/v).The reaction mixture placed into ice cold water and the product was precipitated as pale yellow solid and it was dried and purified by recrystallization from ethanol to give pure product(First Intermediate). 0.025 mole of the above product was dissolved in 15ml of 10% $NaHCO_3$ solution, 0.04 mole (1.5ml) of benzoyl chloride was added, and the reaction mixture was shaken vigorously in an FBF the stopper was removed from time to time since CO_2 evolved.Than dilute HCl was added and the precipitate obtained & recrystallized from ethanol.Thiosemicarbazide (0.01mol) and above product (0.01mol) were taken into the 100ml RBF. The reaction was carried out in ethanol with catalytic amount of glacial acetic acid at refluxed temperature for 4 hr.After completion of the reaction, obtained solid product was filtered and purified by recrystallization from ethanol.

Thin layer chromatography was used to monitor the progress of the reactions. Infrared spectra were recorded on SHIMADZU FT/IR spectrophotometer using KBr pellets at S.G.S.I.T.S,Indore, and values were expressed in cm-1.1HNMR spectra were recorded using a Bruker ADVANCE II 400 NMR spectrophotometer at IIT Bombay and values were reported in ppm downfield from TMS (Tetramethylsilane) as an internal standard. The NMR spectra were obtained in Acetone. The molecular mass of synthesized hybrid was determined by mass spectroscopy. Mass spectra were recorded using CIF Mass Facility IISER Bhopal and results were reported in terms of their m/z values. Melting points were determined by open capillary method.

(Z)-1-((2-(3-chlorophenyl)-1H-benzo[d]imidazol-1-yl (phenyl)methylene) thiosemicarbazide(AD2):

Yellow ; Yield 70%; m.p. 230-232^oC; IR (KBr, cm-1): 3268.52 (N-H, stretch), 2950.15 (C-H,stretch, aromatic), 1647.28 (C=N), 1228.71 (C=S), 1445.71, 1140.07(C-N), 651.00 (C-Cl); ^{1}H-NMR(Acetone): δ ppm: 7.305 (1H, d, Benzimidazol), 2.043

(2H, s, Amine), 3.000(2H, s, CH_2-N), 7.526 (1H, s, H-N, aromatic); the molecular ion peak was not observed but m/z at 398.1 (M-7) and 351.1 fragment peak that confirmed the structure of compound.

(E)-1-((2-(4-(dimethylamino)phenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene)thiosemicarbazide (AD7):

Brown ; Yield 75%; m.p. 242-246^{o}C; IR (KBr, cm^{-1}): 3259.84 (N-H, stretch), 3103.6 (C-H,stretch, aromatic), 1644.39 (C=N), 1287.54 (C=S),1164.09 (C-N); 1H-NMR(Acetone): δ ^{1}H NMR (Acetone-d_6) δ ppm): 7.850 (1H, d, Benzimidazol), 2.037 (2H, s, Amine), 3.160(2H, s, CH_2-N), 7.510 (1H, s, H-N, aromatic), 1.917 (3H, s, -CH_3); the molecular ion peak was not observed but m/z at 406.1 (M-8) and 351.1 fragment peak that confirmed the structure of compound.

(E)-1-((2-(2-chlorobenzyl)-1H-benzo[d]imidazol-1-yl) (phenyl)methylene) thiosemicarbazide(AD8):

Brown;Yield 72%; m.p. 196-198^{o}C; IR (KBr, cm-1): 3369.79 (N-H, stretch), 2972.43 (C-H,stretch, aromatic), 1643.42 (C=N), 1525.76 (N-H, bend), 1166.02(C-N),1287.54 (C=s), 684.76 (C-H, bend, aromatic), 604.71 (C-Cl); 1H-NMR(DMSO): δ ^{1}H NMR (Acetone-d_6) δ ppm): 7.545 (1H, d, Benzimidazol), 2.044 (2H, s, Amine), 7.510 (1H, s, H-N, aromatic), 1.977 (1H, t, -CH); the molecular ion peak was not observed but m/z at 416.9 (M-2).

(E)-1-((2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d] imidazol-1-yl)(phenyl)methylene)thiosemicarbazide (AD10):

Yellow ; Yield 70%; m.p. 190-194^{o}C; IR (KBr, cm^{-1}): 3170.70(N-H, stretch), 2870.30 (C-H,stretch, aromatic), 1645.35 (C=N), 1525.76 (N-H, bend), 1445.71 (C=C, aromatic), 1165.05(C-N), 684.76 (C-H, bend, aromatic),1002.06(O-H); ^{1}H NMR (Acetone) δ ppm: 7.536 (1H, d, Benzimidazol), 2.011 (2H, s, Amine), 7.311 (1H, s, H-N, aromatic), 1.944 (3H, s,); the molecular ion peak was not observed but m/z at 378.1 (M-39) and 351.1 fragment peak that confirmed the structure of compound.

(E)-1-((2-methyl-1H-benzo[d]imidazol-1yl)(phenyl) methylene) thiosemicarbazide(AD13):

Yellow ; Yield 72%; m.p. 220-224^{o}C; IR (KBr, cm^{-1}): 3371.17 (N-H, stretch), 3065.02 (C-H,stretch, aromatic), 1642.42 (C=N), 1445.71 (C=C, aromatic), 1149.9(C-N), 684.76 (C-H, bend, aromatic); ^{1}H NMR (Acetone-d_6) δ ppm): 7.743 (1H, d, Benzimidazol), 2.400(2H, s, Amine), 7.763 (1H, s, H-N, aromatic), 3.415 (2H, d, CH_2-X); the molecular ion peak was not observed but m/z at 304(M+5)confirmed the structure of compound.

(E)-1-((2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene) thiosemicarbazide(AD19):

Yellow ; Yield 78%; m.p. 186-190^{o}C; IR (KBr, cm-1): 3264.66 (N-H, stretch), 2981.4 (C-H,stretch, aromatic), 1619.41 (C=N), 1525.76 (N-H, bend), 1445.71 (C=C, aromatic), 1162.16(C-N), 1285.61(C=S), 684.76 (C-H, bend, aromatic), 2778.5.13 (-OCH_3); ^{1}H NMR (Acetone-d_6) δ ppm): 7.675 (1H, d, Benzimidazol), 2.297 (2H, s, Amine),4.398(1H,s, C=C-H) 7.367 (1H, s, H-N, aromatic), 1.548(3H,t,CH_3); the molecular ion peak was not observed but m/z at 354.3(M-46)and 345.1 that confirmed the structure of compound.

(E)-1-((2-(2,4-dichlorophenyl)-1H-benzo[d]imidazol-1-yl)(phenyl)methylene) thiosemicarbazide(AD20):

Brown ; Yield 75%; m.p. 216-220^{o}C; IR (KBr, cm-1): 3370.75 (N-H, stretch), 2904.92 (C-H,stretch, aromatic), 1620.3 (C=N), 1525.76 (N-H, bend), 1445.71 (C=C, aromatic), 1158.3(C-N), 684.76 (C-H, bend, aromatic),652.13 (C-Cl); ^{1}H NMR (Acetone-d_6) δ ppm): 7.997 (1H, d, Benzimidazol), 2.400 (2H, s, Amine), 3.341(2H, s, CH_2-N), 7.754 (1H, s, H-N, aromatic; the molecular ion peak was not observed but m/z at 475.3 (M+35) and 351.1 fragment peak that confirmed the structure of compound.

2.3 *In vitro* Antimalarial activity:

All the synthesized compounds were screened for *in vitro* antimalarial activity.The *in vitro* antimalarial assay was carried out in 96 well microtiter plates according to the microassay protocol of Rieckmann and coworkers with minor modifications. All the cultures of *P. falciparum* strains were maintained in medium RPMI1640 supplemented with 25m MHEPES, 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 samples, prepared in DMSO and their subsequent dilutions were prepared with culture Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and maintained with 50 % RBCs (O^+). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium, then diluted samples were added to the test wells so as to obtain final concentrations ranging between 0.4µg/ml-100µg/ml in duplicate well containing parasite cell preparation. The culture plates were incubated at 37^{o}C in a candle jar, after 36-40 hours of incubation; thin blood smear slides were prepared from each well and stained with JSB stain. The slides were observed under microscope to record maturation of ring stage parasites 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 IC_{50} value of test compounds.

3. Results and discussion

3.1 Docking studies:

The result of the extra precision docking experiments of all the designed compounds along with standard drug is summarized in table 2. The X-ray crystallographic structure of PfENR (PDB ID: 3AM5) were obtained from protein data bank through internet. Inspection of Thiosemicarbazone-benzimidazole hybrids in the active site of enzyme revealed hydrogen bonding and π-π interaction with the residues ASN 218, ALA217, LEU216,SER111, GLY106, and LEU164.

All the designed compounds revealed molecular interaction into the active site of enzyme. The observed interaction of compound AD13 into the active site of PfENR enzyme are shown in fig 1 (a), (b) and (c). The good binding interaction of compound with enzyme explains highest antimalarial

S. No.	Com. code	Docking score	Glide Emodel energy	RMSD	Interacting amino acid	IC50 value
1	AD1	-7.777	-68.120	0.018	Gly 106, THR 108, ASP 107	-
2	AD2*	**-8.067**	-74.315	0.004	THR 108, ASP 107	0.45
3	AD3	-7.529	-55.381	0.003	ALA 219, SER 319	-
4	AD4	-7.964	-65.921	0.005	SER 216, SER 317	-
5	AD5	-8.598	-58.361	0.010	ALA 217, ASN 218	-
6	AD6	-9.012	-75.354	0.023	Gly 106, Ile 105, Gly 104	-
7	AD7*	**-8.280**	-65.923	0.001	ASN 216, ALA 217	0.35
8	AD8*	**-9.102**	-95.623	0.016	SER 215, ALA 217	1.25
9	AD9	-9.027	-73.750	0.015	SER 215, LEU 216, ALA 217	-
10	AD10*	-10.217	-61.390	0.008	SER 215, ASN 218, Leu 216	0.20
11	AD11	-8.384	-67.280	0.005	ALA 217, ASN 218,	-
12	AD12	-10.338	-64.656	0'008	SER 317, ARG 318	-
13	**AD13***	**-10.572**	**-79.976**	0.027	ALA 319, SER 317, ALA320	0.06
14	AD14	-8.060	-54.782	0.013	ALA 217, ASN 216	-
15	AD15	-9.410	-58.168	0.046	ASN 218, ALA 217	-
16	AD16	-7.776	-51.286	0.049	ALA 217, ASN 218	-
17	AD17	-8.907	-68.103	0.045	SER 215, LEU 216, ALA 217	-
18	AD18	-8.892	-68.734	0.004	ALA 217, ASN 218,ILE 105	-
19	AD19*	-8.737	-56.558	0.018	SER 216, LEU 216, ASN 218	0.72
20	AD20*	-8.887	-65.124	0.007	ASN 218, ALA 217, leu 216	0.98
21	CLQ	-7.021	-52.231	0.002	SER 216, LEU 216	0.02
22	QUE	-	-	-	-	0.268

Table 2: Compound code, Glide score, Emodel energy, RMSD, amino acid interactions and IC50 values of compounds and standard with PfENR

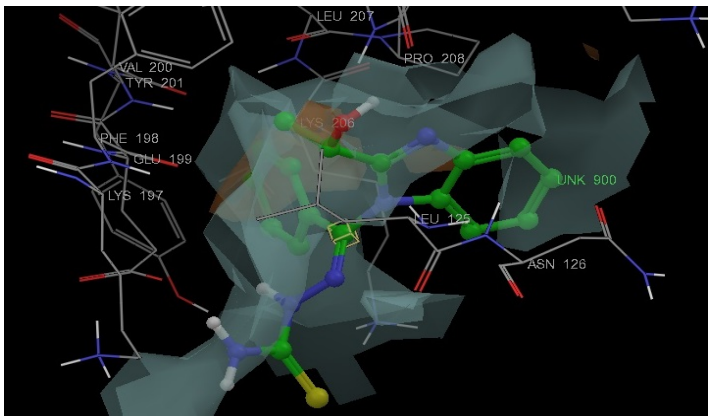
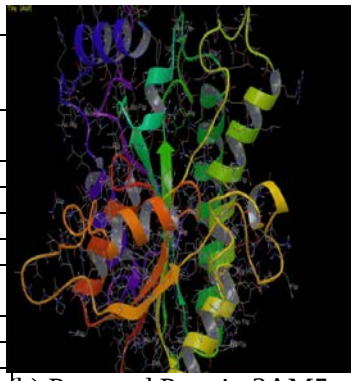
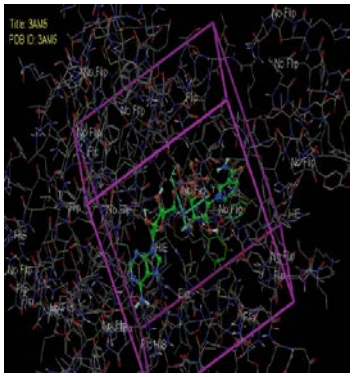


Fig no 1.-(a) Best binding Pose AD13



(b) Prepared Protein 3AM5



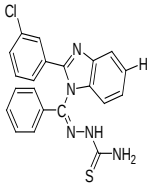
(c) Grid generation

3.2 Synthesis:

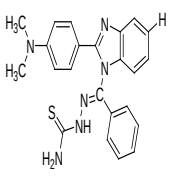
Synthesis of thiosemicarbazone-benzimidazole hybrids were carried out as outlined in reaction scheme 1. thiosemicarbazone-benzimidazole hybrid derivatives seven compound (AD2, AD7, AD8, AD10, AD13,AD19 and AD20) were synthesized by reaction of *o*-phenylene diamine with substituted aldehydes and acids in the presence of ethanol and NH_4Cl (30 mol%). The resulting mixture was stirred for 2 hr at 80^oC. The reaction mixture placed into ice cold water and the product was precipitated as pale yellow solid and it was dried and purified by recrystallization from ethanol to give pure product(3).Above product was dissolved in 10% $NaHCO_3$ solution and benzoyl chloride was added and the reaction mixture was shaken vigorously in an FBF the stopper was removed from time to time since CO_2 evolved. Than dilute HCl was added and the precipitate obtained & recrystallized from ethanol(5). Thiosemicarbazide and above product were taken into the 100ml RBF. The reaction was carried out in ethanol with catalytic amount of glacial acetic acid and refluxed for 4 hr. After completion of the reaction, obtained solid product(7) was filtered and purified by recrystallization from ethanol. The completion of the reaction was confirmed by TLC, ethylacetate:hexane, (2:1 v/v).

3.3 *In vitro* Antimalarial activity:

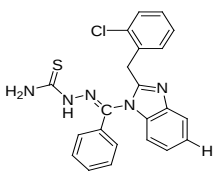
All the seven synthesized hybrids were evaluated for their antimalarial activity against chloroquine resistant strain of *P. Falciparum* using chloroquine and quinine as reference drugs (Table 2). In order to get structural insight, two point variations were made in the thiosemicarbazone–benzimidazole hybrids (AD2,AD7,AD8,AD10,AD13, AD19, and AD20). In the structural motive of these hybrids, the Thiosemicarbazone were kept common and variation was made in benzimidazole ring. Among these hybrids, compound AD13 was found to be active against chloroquine sensitive strain with IC50 value 0.06 µg/ml. The activity of compound AD13 was found to be comparable with chloroquine and better than the quinine (Table 2).



AD2



AD7



AD8

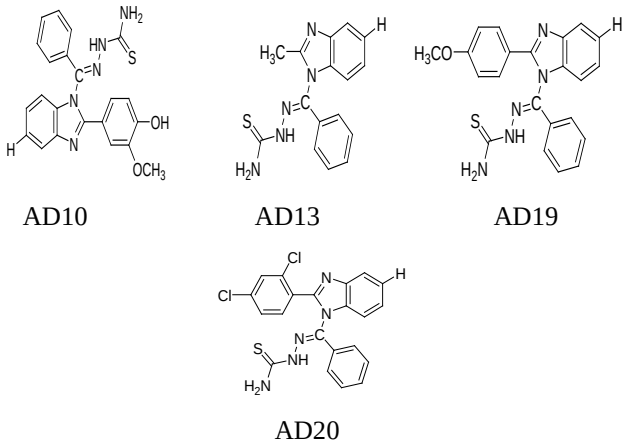


Fig.2-Structure of synthesized compounds

4. Conclusion

In the present study, have reported docking, synthesis and antimalarial activity of series of thiosemicarbazone-benzimidazole hybrids. XP Glide docking scores and docking poses of designed compounds and standard suggest that these compounds adopt similar binding mode with active site residue of PfENR (3AM5) as hydrogen bond, hydrophobic and π-π stacking interactions, which help in the stabilization of drug in active site. The *in vitro* evaluation of synthesized hybrids against chloroquine sensitive strain of *P. falciparum* depicted activity in nanomolar range. The compound AD13 exhibited comparable antimalarial activity with chloroquine and better activity than the quinine. Antimalarial activity of other compounds was comparable to the standard drugs. Docking pattern in the *P. falciparum* PfENR described in present study and good *in vitro* antimalarial activity exhibited by the thiosemicarbazone-benzimidazole hybrids and reveals that in near future, they could be developed as lead for antimalarial compounds.

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