Original Article

# Insilco Study, Synthesis Of Thiazole Molecules As Possible Dihydrofolate Reductase Inhibitors Against Malaria

**Alok Yadav[1], Preeti Verma[2*], Bhupendra Chauhan[3*], Abhinav Prasoon Mishra[4], Peeyush[5], anju singh[6]**

[1, 2*, 4] Department of Pharmaceutical chemistry, Advance Institute of Biotech & Paramedical Sciences, Kanpur, Uttar Pradesh, India.
[2, 3*, 5] Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University Gangoh, Saharanpur, Uttar Pradesh, India.
[6] University Institute of Pharmacy, Chhatrapati Shahu Ji Maharaj University, Kanpur, U.P.
[5] Maharana Pratap College of Pharmacy, Kothi, Mandhana Kanpur - 209217, Uttar Pradesh, India.
***Corresponding Author:** - Preeti Verma, Bhupendra Chauhan
*Phd Research Scholar, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University Gangoh, Saharanpur, Uttar Pradesh, India, Email ID: Verma3preeti@gmail.com
*Associate Professor, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University Gangoh, Saharanpur - 247341, Uttar Pradesh, India, E-mail: bhupendrapharma@gmail.com
DOI: 10.47750/pnr.2023.14.03.247

## Abstract

Heterocyclic compounds are the main class of medicinally important compounds. Many heterocyclic compounds bearing a five-membered ring in their structure have a good spectrum of biological activities. In medicinal chemistry, thiazole and its analogues are promising scaffolds. Many of them have been reported to exhibit a variety of biological responses, including anti-hyperlipidemic, anti-HIV, anticancer, anti-Alzheimer, antihypertensive, anti-inflammatory, antimicrobial, anticonvulsant, antiviral, antimalarial, and antidiabetic activities. In this study a modern approach has been undertaken to identify new hits of thiazole derivatives as antimalarials targeting against *Pf*-DHFR. It has been conceived, synthesised, and tested for potential anti-malarial action to create a number of new thiazole derivatives. The antimalarial activity of the synthesized thiazole derivatives was assessed against human pathogenic malarial strain viz. Plasmodium falciparum while quinine was taken as the standard drug. compound 1a-1e was found to be most promising which exhibited strongest inhibitory activity against *Pf*-DHFR which was higher than the reference drug quinine. The aim of this research is to identify and try making a SAR (Structure Activity Relationship) of substituted thiazole nucleus as possible new antimalarials as shown in compound 1a-1e. The result of molecular docking studies showed that compounds 1a, 1b, 1c, 1d and 1e showed good docking scores with protein (protein ID-7CTZ). The compounds with the greatest docking scores were 1a and 1b (-9.9 and -9.8). Contrarily, compounds 1a, 1b, 1c, 1d, and 1e displayed favorable docking results and important interactions with amino acid residues such Gly150, leu146, Phe197, Val198, Asp145, Asp148, Thr152, Thr149, Glu144, and Ala178. The results of biological activity and docking studies showed that an electron-withdrawing group at the fourth position of the attached phenyl ring of thiazole derivatives is essential for better anti-malarial activity and a favorable drug-like profile that can lead to the emergence of a potential drug molecule in further development.

**Keywords:** Thiazole derivatives, anti-malarial activity, ADME study, Docking and synthesis.

## 1. INTRODUTION

Malaria represents a considerable threat to public health, not only in the past, but also in recent period where the disease was reported to be responsible for approximately 405,000 death worldwide. The etiologies of malaria is explored due to the development of protozoan parasite of the genus plasmodium in erythrocytes through a bite of female anopheles mosquito.*Plasmodium falciparum* dihydrofolate reductase *(Pf*-DHFR) is an essential enzyme in the folate pathway and is an important target for antimalarial drug discovery. In this study, a modern approach has been undertaken to identify new hits of thiazole derivatives as antimalarials targeting *Pf*-DHFR. This study contributes to anti-malarial research effort by conducting *in silico* assessment of 231 compounds against plasmodium falciparum dihydrofolate enzyme for potential inhibition malarial activity **[1,2]**. According to literature studies, it is well recognized that *Plasmodium falciparum* and *P. vivax* (accounts for 99.7% of all cases in 2023) have developed resistance to nearly all the currently available antimalarial drugs, such as quinine, sulfadoxine/pyrimethamine, mefloquine and halofantrine, and, thus posing significant threat to malarial control and results in increased malarial morbidity and mortality **[3, 4]**

### 1.1 Microbiology Of Anopheline Mosquito

The female anopheline mosquito is the vector for Plasmodium spp. When the mosquito takes a blood meal, sporozoites contained in the salivary glands are discharged into the puncture wound.14 Within approximately 60 min, the sporozoites are carried via the blood to the liver hepatocytes, thus initiating the pre erythrocytic or primary exoerythrocytic cycle **[5]**.

### 1.2 Life Cycle Of Malaria

Human malaria infection is initiated when a female Anopheline mosquito injects Plasmodium sporozoites into the skin during a blood meal. Sporozoites actively reach peripheral circulation and migrate to the liver in which they replicate

within hepatocytes forming merozoites that are released into the bloodstream. Merozoites invade red blood cells (RBCs) and develop through ring, trophozoite, and schizont stages before forming new merozoites that are released at schizont egress and reinvade new RBCs. A small proportion of blood stage parasites develop into sexual stages called gametocytes that reach the dermis where they are taken up by another mosquito. After fertilization and sporogonic development in the mosquito midgut, infectious sporozoites are formed that reach the salivary glands for transmission into another host. (B) Schematic representation of P. falciparum gametocyte developmental stages. Gametocytes undergo five distinct morphological stages during development. Stage I and early stage II are morphologically similar to early stage asexual parasites, and late stage II is the first stage that can be distinguished from asexual trophozoites. Late stage III and stage IV are further elongated and characterized by their spindle shape, whereas in stage V gametocytes, the ends are more rounded forming a crescent shape with minimal visible host cell surface **[6]**.

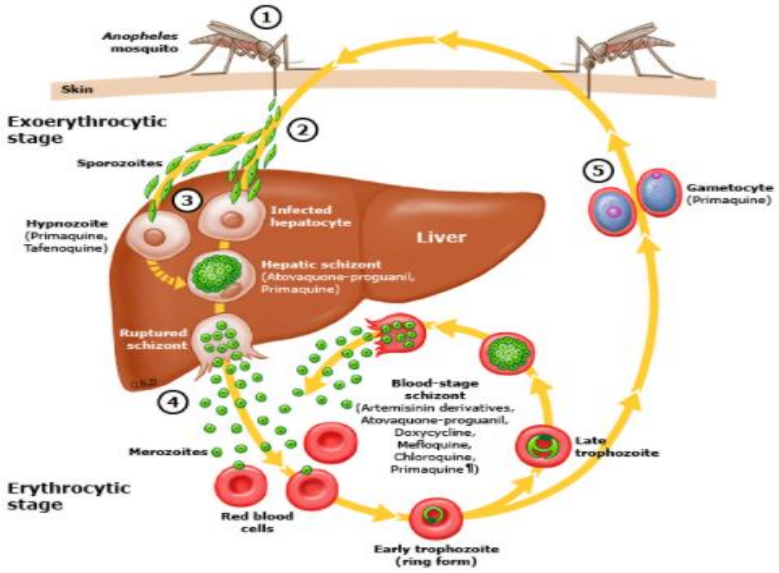



**Fig: 1** Life cycle of malaria

## 2. MATERIALS AND METHODS

### 2.1 Protien preparation

The protein was download from the Protein Data Bank (PDB). Protein crystal structures are often planned well in advance of docking by performing extra procedures not covered by the x-ray crystal structure refining process and 3-D structure of PFDHFR (falciparum dihydrofolate reductase) **[7]**.

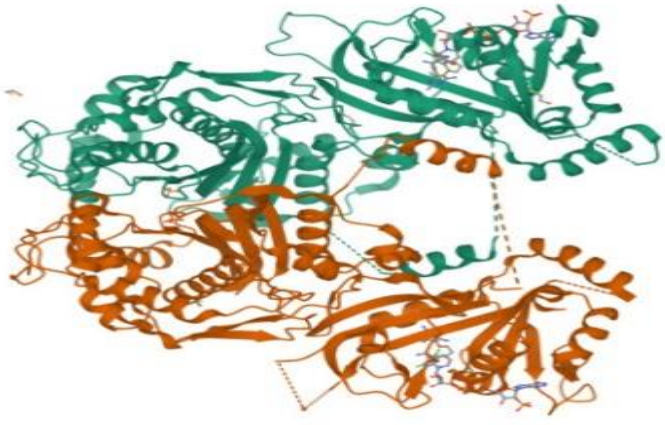


**Fig: 2** Protein structure of falciparum dihydrofolate reductase (***PF*DHFR**)

### 2.2 Prediction of binding site

Protein binding and active sites are often found in structural pockets and voids. It facilitates in the identification and quantification of surface-accessible areas as well as interior inaccessible cavities for PFDHFR. The volume of cavities included was calculated using the MVD tool. It assists in the identification and quantification of surface-accessible regions as well as integrand PFDHFR aids in the identification and quantification of surface-accessible regions as well as inter. The top five cavities were predicted using PFDHFR, which assists in locating and characterising surface-accessible areas. Having a capacity of 170.78 represents the biggest cavity (cavity-1). Typically, the cavity with the highest volume is linked to the binding site. The PFDHFR binds assists in the identification and quantification of surface-accessible areas

and is associated with the biggest cavity, giving a strong backdrop for cavity 1 to operate as a binding site. The largest cavity was linked with amino acids like Gly150, leu146, Phe197, Val198, Asp145, Asp148, Thr152, Thr149, Glu144, Ala178.

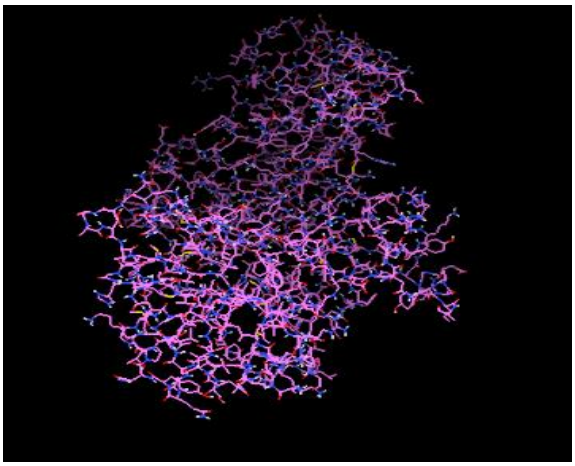


**Fig: 3** Binding site prediction in PFDHFR

### 2.3 ADMET study

The library of 231 compounds and two reference drugs was selected for calculating the ADMET (absorption, distribution, metabolism, excretion and toxicity) using Discovery studio 3.1 (. The ADMET descriptors methodology was used to predict the values of the descriptors Absorption, Solubility, Hepatotoxicity, Plasma protein binding (PPB) **[8]** using descriptors AlogP98 and 2D polar surface area (PSA 2D). A model developed by Cheng and Merz with R2 = 0.78 was used to predict aqueous solubility. All of the models used to predict ADME and toxicity have strong R2 values and are capable of showing confidence ellipses **(Fig. 4)**. Compounds discovered outside the 94% and 98% ellipse regions were poorly absorbed (30% absorbed) **[9]**. As a result of this, As a result, the compound library had dropped 231. Furthermore, compounds having poor (2) and very low (3) human intestinal absorption, extremely low (0) or no drug similarity (5), hepatotoxicity (1), and 90% plasma protein binding (0) were excluded. In comparison to marked artimisnin, the reference compounds 1a, 1b, 1c, 1d, and 1e demonstrated antimalarial activity. The planned library has dropped from 231 compounds, which were used to predict antimalarial activity once more.

### 2.4 Docking

Docking was done using the protein data bank crystal structures of natural and mutant Pf-DHFR complexes (7CTZ). Water molecules and co-crystallized ligands 1a, 1b, 1c, 1d, and 1e were removed from the protein. For the flexible docking study, the Python molecular viewer (PMV) was employed **[10]**. Both the protein and the ligand must have a three-dimensional structure for PMV to work. A docking simulation study was conducted to determine the ligand's inhibitory capability against the Pf-DHFR enzyme. The target Pf-DHFR was docked with compound thiazole derivative. The goal was to assess the relative affinities and binding interactions of inhibitors for the target. Thiazole binds to the Gly150, leu146, Phe197, Val198, Asp145, Asp148, Thr152, Thr149, Glu144, Ala178 residues of Pf-DHFR with a high affinity (-9.9). It also has hydrogen bond energy (-6-9 kcal/mol) and makes three hydrogen bonds with Ala178 Glu134 leu146 Asp145. Was shown in table 1.Thiazole has a higher affinity for *Plasmodium falciparum*'s HGPRT target.

### 2.5 Synthesis

All of the chemicals and solvents utilised in the synthesis, recrystallization, and analysis were of AR grade and were not further purified. The melting points of the synthesised compounds were determined using a Melting Point device (Melting Point M560) at a temperature gradient of 10 C/min. The UV-Spectra (lmax) of the synthesised compounds were captured using a Shimadzu UV1800 UV-VIS spectrophotometer. On a Bruker ALPHA FTIR spectrometer, the FT-IR spectra of the produced compounds were recorded. The 1 H-NMR spectra of the synthesised compounds were recorded at 300 MHz in DMSO using a Bruker Avance DPX 300 NMR spectrometer, and the 13C-NMR spectra were likewise obtained in DMSO using a Bruker Avance DPX 100 NMR spectrometer. As an ionisation approach, the mass spectra of the produced compounds were recorded using a ZQ-4000 equipped with an Electrospray Ionizer. The intermediates (3) and (5) were synthesised using previously published processes indicated in Scheme 1 **[11, 12]**.

## 3. RESULTS AND DISCUSSION

**Table 1:** Docking result of thiazole derivatives with PFDHFR

| Compound | Mol dock Score | Hydrogen Bond | Residue in interaction | H bonding Residue | H Bonds |
|---|---|---|---|---|---|
| Thiazole with PFDHFR | -9.9 | -6.9 | Gly150, leu146, Phe197, Val198, Asp145, Asp148, Thr152, Thr149, Glu144, Ala178. | Ala178 Glu134 leu146 Asp145 | 4 |

**Table: 2** Confirmers of thiazole derivatives with PFDHFR after docking (1a)

| Mode | Affinity (kcal/mol) | dist from best mode rmsd l.b. |
|---|---|---|
| 1 | -9.9 | 0.000 |
| 2 | -6.9 | 0.921 |
| 3 | -6.9 | 0.949 |
| 4 | -5.6 | 1.423 |
| 5 | -7.5 | 1.669 |
| 6 | -5.3 | 1.870 |
| 7 | -5.0 | 56.729 |
| 8 | -4.1 | 26.729 |
| 9 | -5.9 | 1.876 |
| 10 | -7.9 | 56.786 |
| 11 | -3.8 | 28.845 |
| 12 | -2.7 | 32.438 |
| 13 | 3.8 | 36.139 |
| 14 | 3.8 | 12.712 |
| 15 | 3.8 | 14.327 |

**Table: 3** Confirmers of thiazole derivatives with PFDHFR after docking (1b)

| Mode | Affinity (kcal/mol) | dist from best mode rmsd l.b. |
|---|---|---|
| 1 | -9.8 | 0.000 |
| 2 | -5.8 | 0.999 |
| 3 | -5.9 | 0.987 |
| 4 | -5.6 | 1.145 |
| 5 | -5.5 | 1.543 |
| 6 | -5.1 | 37.061 |
| 7 | -5.0 | 37.008 |
| 8 | -4.9 | 36.965 |
| 9 | -4.9 | 1.778 |
| 10 | -4.9 | 1.348 |
| 11 | -4.8 | 1.926 |
| 12 | -4.7 | 36.965 |
| 13 | -4.7 | 39.403 |
| 14 | -4.7 | 36.990 |
| 15 | -4.7 | 36.965 |

**Table: 4** Confirmers of thiazole derivatives with PFDHFR after docking (1c)

| Mode | Affinity (kcal/mol) | dist from best mode rmsd l.b. |
|---|---|---|
| 1 | -8.9 | 0.000 |
| 2 | -5.9 | 0.022 |
| 3 | -5.8 | 0.932 |
| 4 | -5.7 | 0.999 |
| 5 | -5.6 | 1.345 |
| 6 | -5.3 | 23.184 |
| 7 | -5.2 | 1.937 |
| 8 | -5.4 | 1.757 |
| 9 | -5.2 | 1.964 |
| 10 | -4.9 | 36.636 |
| 11 | -4.9 | 33.133 |
| 12 | -4.7 | 25.134 |
| 13 | -3.7 | 25.184 |
| 14 | -3.6 | 53.164 |
| 15 | -2.6 | 43.154 |

**Table: 5** Confirmers of thiazole derivatives with PFDHFR after docking (1d)

| Mode | Affinity (kcal/mol) | dist from best mode rmsd l.b. |
|---|---|---|
| 1 | -8.3 | 0.000 |
| 2 | -6.0 | 0.995 |
| 3 | -5.9 | 1.398 |
| 4 | -5.8 | 1.268 |
| 5 | -5.7 | 1.300 |
| 6 | -5.6 | 0.953 |

| | | |
|---|---|---|
| 7 | -5.3 | 28.960 |
| 8 | -5.3 | 1.972 |
| 9 | -5.2 | 1.804 |
| 10 | -3.9 | 29.230 |
| 11 | -4.8 | 36.938 |
| 12 | -4.9 | 29.230 |
| 13 | -4.9 | 31.938 |
| 14 | -4.8 | 36.652 |
| **15** | **-4.8** | **13.875** |

**Table: 6** Confirmers of thiazole derivatives with PFDHFR after docking (1e)

| Mode | Affinity (kcal/mol) | dist from best mode rmsd l.b. |
|---|---|---|
| 1 | -8.1 | 0.000 |
| 2 | -6.0 | 0.999 |
| 3 | -5.9 | 1.435 |
| 4 | -5.8 | 1.324 |
| 5 | -5.7 | 1.321 |
| 6 | -5.6 | 0.953 |
| 7 | -5.3 | 38.960 |
| 8 | -5.3 | 1.972 |
| 9 | -5.2 | 1.804 |
| 10 | -4.9 | 29.230 |
| 11 | -4.8 | 37.938 |
| 12 | -4.8 | 36.652 |
| 13 | -4.8 | 38.938 |
| 14 | -4.7 | 37.654 |
| 15 | -4.7 | 13.875 |

### 3.1 Pharmacophore based screening

We used the "Adjustable" fitment approach and the "Ideal" conform creation choice in the "pharmacophore" procedures of the DS software to carry these out database finding studies.

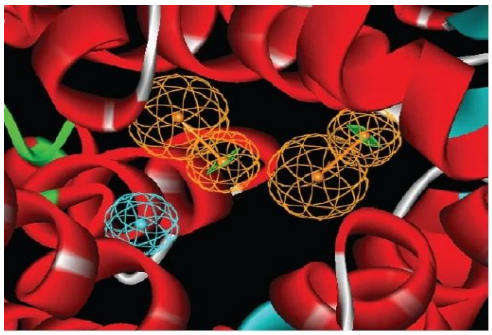


**Fig: 4** Pharmocophore structure

The compounds from the thaizole-based chemical database that matched most of the pharmacophore model's characteristics had been kept as hits. High fit ratings, which suggest great matches, were the parameter for screening for further confirmation **[13]**.

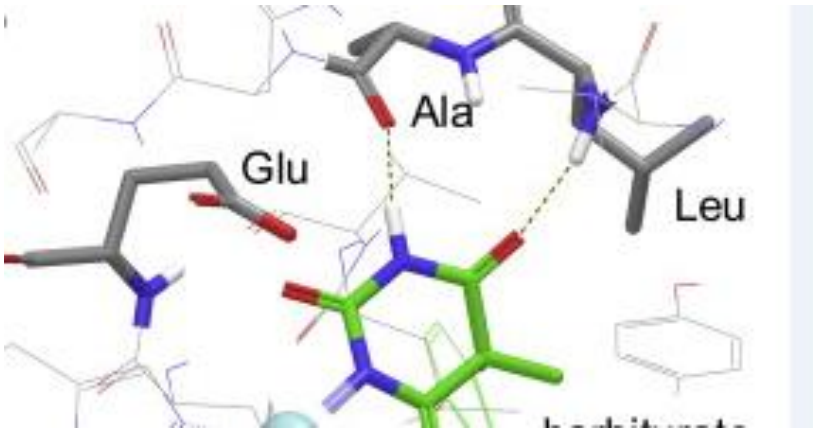



**Fig: 5** Analytical structure of thiazole

### 3.2 ADME Prediction

The QikProp tool was used to predict the pharmacokinetic parameters of the screened hit compounds in silico. Owing to poor ADME (absorption, distribution, metabolism, and excretion) qualities, over half of in clinical trials, every therapeutic candidate fails. To proceed with virtual screening, **[14]** the complete set of retrieved hits was filtered using Lipinski's rule and ADMET attributes.

### 3.3 General procedure for the synthesis for thiazole derivatives (1a-1e)

To a solution of 1 equivalent of thiosemicarbazone in isopropyl alcohol was added in 1 equivalent of phenacyl bromide. The resulting mixture was kept under reflux for 5 hours. After cooling at room temperature, the formed precipitates was filtered and washed with saturated solution of NaHCO3 followed by cold distilled water. Final product was recrystallized in ethanol. the purity of the product as well as composition of reaction mixture were monitored by TLC (Thin layer chromatography) using solvent n-hexane:ethyl acetate(3:2) **[15]**.

#### 3.3.1.3-methoxy-2-{2-[1-(4-methoxylphenyl)ethyl]hydrazinylidene}-4-phenyl-2,3-dihydro-1,3-thiazole (1a)

Cream precipitates; Yield 75%; mp. 180–181°C; Rf 0.5; IR (KBr) Vmax(cm-1 ): 1121cm-1 (C-N), 1513 cm-1 (C = C), 1258cm-1 (OCH3), 3050cm-1 (C-H Aromatic), 3295cm-1 (N-H). 1HNMR(300 MHZ CDCl3 ) :δ (ppm)9.88 [s,1H,CH]; 7.38–7.47 [m,Ar-H]; 6.25–7.38[s,4H,Ar-H]; 6.49 [s, 3H,thiazole]; 4.02 [s,O-CH3]; 3.84 [s,-CH3]. 13CNMR (75 MHZ, CDCl3): δ 160.65, 151.06, 142.11, 134.92, 128.82, 128.09, 126.93, 126.37, 114.31, 113.92, 103.19, 55.32. MS (EI) m/z323.41 (M+ +1).

#### 3.3.2. 2-{2-[1-(4-ethoxylphenyl)methyl]hydrazinylidene}-4-phenyl-2,3-dihydro-1,3-thiazole

**(1b)**Yellow precipitates; Yield 71%; mp. 206–207°C; Rf 0.73. IR (KBr) Vmax(cm-1 ): 1132 cm-1 (C-N), 1513 cm1 (C= C), 3050 cm-1 (C-H Aromatic stretching), 3127cm-1 (N-H stretch secondary amine), 3400 cm-1 (OH stretching). 1HNMR (300 MHZ CDCl3) : δ (ppm) 8.74 [s,1H,CH]; 6.86–7.81 [m, Ar-H]; 7.02 [ s, 1H, NH]; 6.54 [s, 1H, thi azole]; 5.50 [s, 1H, OH]; 2.24[ s, 1H, CH3]. MS (EI) m/z 311.11 (M+ +1).

#### 3.3.3. 2-{2-[1-(4-ethoxylphenyl)ethyl]hydrazinylidene}-4-phenyl-2,3-dihydro-1,3-thiazole

**(1c)** Light brown precipitates; Yield 62%; mp. 210–212°C; Rf 0.6 was also determined IR (KBr) Vmax(cm− 1 ): 620 cm− 1 (C-S), 1168 cm− 1 (C-N), 1606 cm− 1 (C= C), 3050 cm− 1 (C-H Aromatic), 3127 cm− 1 (N-H), 3416 cm− 1 (OH).

#### 3.3.4. 2-{2-[1-(4-hydroxyphenyl)ethyl]hydrazinylidene}-4-phenyl-2,3-dihydro-1,3-thiazole

**(1d)** Light green precipitates; Yield 69%; mp. 200–202°C, Rf 0.68 was also determined. IR (KBr) Vmax(cm-1 ): 1226 cm-1 (C-N), 2228 cm-1 (C = N), 3108 cm-1 (C-H Aromatic stretching), 3400 cm-1 (OH), 3127 cm-1 (N-H).

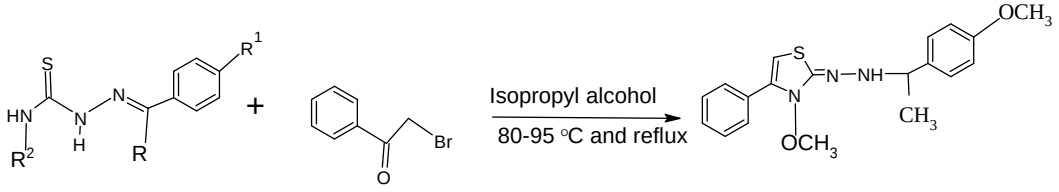
#### 3.3.5.3-methoxy-2-{2-[1-(4-hydroxylphenyl)ethyl]hydrazinylidene}-4-phenyl-2,3-dihydro-1,3-thiazole (1e)

Blackish brown precipitates; Yield 65%; mp. 198–199°C; Rf 0.66. IR (KBr) Vmax(cm− 1 ): 1230 cm− 1 (C-N), 1599 cm− 1 (C= C), 3434 cm− 1 (OH), 3295 (N-H stretching).

**Table: 7** Molecular descriptors of thiazole derivatives.

| Compound name | R | R1 | R2 | Yield | Melting point |
|---|---|---|---|---|---|
| **1A** | CH3 | OCH3 | OCH3 | 67% | 170–171ºC |
| **1B** | H | OC2H5 | H | 69% | 201–208 ºC |
| **1C** | CH3 | OC2H5 | H | 76% | 211–213ºC |
| **1D** | CH3 | OH | H | 83% | 194–198ºC |
| **1E** | CH3 | OH | CH3 | 81% | 212–223 ºC |

### Syhesis scheme 1




Thiosemicarbazone derivatives | Phenacyl bromide | Subsituted thiazole derivatives

**Table: 8** Thiazole derivatives.

| Compound name | R | R1 | R2 |
|---|---|---|---|
| 1A | CH3 | OCH3 | OCH3 |
| 1B | H | OC2H5 | H |

| | | | |
|---|---|---|---|
| 1C | CH3 | OC2H5 | H |
| 1D | CH3 | OH | H |
| 1E | CH3 | OH | CH3 |

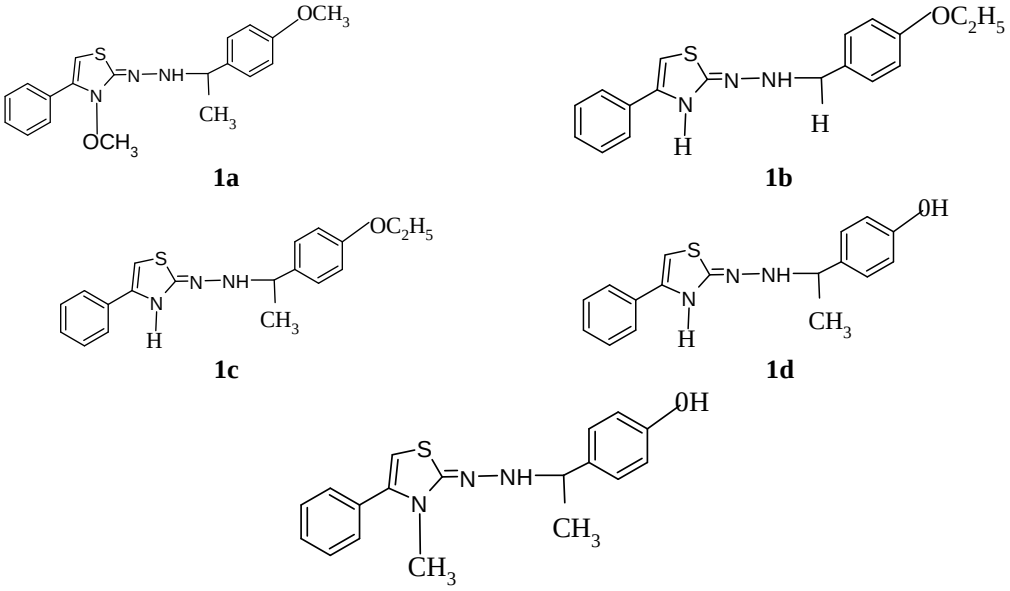



**Fig: 6** Chemical structures of thiazole derivatives

## 4. IN VITRO ANTIMALARIAL ACTIVITY SCREENING

The synthesized thiazole derivatives (1a-1e) were evaluated for their anti-malarial activity and their results was expressed in terms of IC50 µg/ml . The in vitro anti-malarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann and coworkers with minor modifications. The cultures of Plasmodium falciparum strain were maintained in RPMI-1640 medium supplemented with 25mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of Plasmodium 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 200µl 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+). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and dilutions were prepared with culture medium. The diluted samples in 20µl volume were added to the test wells so as to obtain final concentrations (at fivefold dilutions) ranging between 0.4µg/mL to 100µg/mL in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37˚C in a candle jar. After 36 to 40h 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 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 minimum inhibitory concentrations (MICs). Chloroquine was used as the reference drug **[16, 17]**.

## 5. CONCLUSION

In conclusion, the present study shown the synthesis of hybrid thiazole and their antimalarial evaluation against choroquine resistant Dd2 strain of P. falciparum. The result suggested, that compounds 1a and 1b as most potent derivatives among the tested compounds with significant in vitro antimalarial activity and may serve as lead for identifying new class of Pf-DHFR inhibitor and found to be most promising which exhibited strongest inhibitory activity against P.falciparum The result of molecular docking studies showed that compounds 1a-1e has crystal alignment as crystal ligand of protein(PDB ID : 7CTZ) and compound 1a and 1b showed highest docking score such as (-9.9 and -9.8) Therefore, compounds 1c, 1d and 1e also showed highest docking Score(-8.9, -8.3, -8.1 respectively) and critical interactions with Ala178, Glu134, leu146, Asp145. The results of biological activity and docking study revealed that the presence of electron withdrawing group at 4th position of phenyl ring attached is crucial for better anti-malarial activity and favorable drug-like profile which can emerge as a potential drug molecule in further development. In conclusion, the structural features of compound 1a and 1b may be considered for the development of newer anti-malarial agents.

## REFERENCES


1. Lynne S Garcia. Malaria.Clin Lab Med. 2010; 30(1): 93–129.
2. José Antonio Garrido-Cardenas, Lilia González-Cerón, Francisco Manzano-Agugliaro, Concepción Mesa-Valle. Plasmodium genomics: an approach for learning about and ending human malaria, genetics, evolution, and phylogeny – review, 2018; 00436-018-6127-9.
3. White NJ. Plasmodium knowlesi: the fifth human malaria parasite. Clin Infect Dis. 2008; 46(2): 172–173.
4. Lichtenberg FR. Pharmaceutical innovation and the burden of disease in developing and developed countries. New York: Columbia University and the National Bureau of Economic Research; 2005.
5. Craft JC. Challenges facing drug development for malaria. Curr Opin Microbiol 2008;11(5):428–33.

6. White MT, Conteh L, Cibulskis R, Ghani AC. Costs and cost-effectiveness of malaria control interventions—a systematic review. Malar J 2011;10:337.
7. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant Plasmodium vivax: a systematic review and metaanalysis. Lancet Infect Dis 2014;14:982–91.
8. Hanboonkunupakarn B, White NJ. The threat of antimalarial drug resistance. Trop Dis Travel Med Vaccines 2016;2:10.
9. Ashley EA, Phyo AP. Drugs in development for malaria. Drugs 2018;78 (9):861–79.
10. Schuhmacher A, Gassmann O, Hinder M. Changing R&D models in researchbased pharmaceutical companies. J Transl Med 2016;14:105.
11. Anvikar AR, Sharma B, Sharma SK, Ghosh SK, Bhatt RM, Kumar A, et al. In vitro assessment of drug resistance in Plasmodium falciparum in five States of India. Indian J Med Res 2012;135:494–9.
12. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 2009;361:455–67.
13. Nzila A. Inhibitors of de novo folate enzymes in Plasmodium falciparum. Drug Discov Today 2006;11:939–44.
14. Gahtori P, Pandey R, Kumar V, Ghosh SK, Das A, Kalita JM, et al. Toward resistance-compromised DHFR inhibitors part 1: combined structure/ligand-based virtual screenings and ADME-Tox profiling. J Chemom 2016;30:462–81.
15. Toyoda T, Brobey RK, Sano G, Horii T, Tomioka N, Itai A. Lead discovery of inhibitors of the dihydrofolate reductase domain of Plasmodium falciparum dihydrofolate reductasethymidylate synthase. Biochem Biophys Res Commun 1997;235:515–9.
16. Sahu S, Ghosh SK, Kalita JM, Dutta M, Bhat HR. Design, synthesis and antimalarial screening of some hybrid 4-aminoquinoline-triazine derivatives against pf-DHFR-TS. Exp Parasitol 2016;163:45–9.
17. Sahu S, Ghosh SK, Ghoshal A, Kalita J, Gahtori P, Bhattacharyya DR. Microwave assisted synthesis, antimalarial screening and structure–activity-relationship exploration of some phenylthiazolyl-triazine derivatives against dihydrofolate reductase. Med Chem Res 2016;25:2916–23.