Design, Synthesis and Molecular Modeling Studies of Thiosemicarbazone & Thiazole Derivatives as Potential Anti-Malarial Agents

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Research Article

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Abstract

A series of novel thiosemicarbazone & thiazole derivatives (Kp1-10) have been designed, synthesized and evaluated for potential anti-malarial activity. The antimalarial activity of the synthesized thiazole derivatives (Kp1-10) was assessed against human pathogenic malarial strain viz. Plasmodium falciparum while quinine was taken as the standard drug. Compound Kp-9 was found to be most promising which exhibited strongest inhibitory activity against P. falciparum with an IC\textsubscript{50} value of 0.29\(\mu\)g/mL which was higher than the reference drug quinine (1.26\(\mu\)g/mL). The SAR study revealed that the substitution with electron withdrawing group at phenyl increases anti-malarial activity as shown in compound Kp-9. The result of molecular docking studies showed that compounds Kp-9, Kp-1, Kp-3, Kp-4 showed good docking scores with protein (PDB code: 5TBO). The compound Kp-9 showed highest docking score (-9.519). Whereas, compounds Kp-1, Kp-3, Kp-4 and Kp-10 showed good docking scores (-8.764, -8.406, -9.062, -9.435 respectively) with critical interactions with the amino acid residues such as VAL532, ILE237, LEU531, HIE185, TYR528, ASN274, ARG265. 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.

1. Introduction

Protozoan parasite existence was discovered even in millions of years old fossil sponges. Protozoan parasites are harmful to humans and cause one million deaths yearly [1]. While there is little doubt that the protozoan disease burden is focused in tropical and subtropical regions of the world, more temperate areas of our globe, including North America and the Asia Pacific region, are also influenced by protozoan diseases such as Malaria, Leishmaniasis, Filariasis, Cryptosporidiosis, African trypanosomiasis, Chagas disease[2]. Here, we only focused on malaria which is a life threatening disease. According to World health organization (WHO) 219 million cases of malaria and 435,000 related deaths occurred in 2017 in the world malaria report 2018. India apparently accounts for 4% of the worldwide malaria burden and represents 87% of Southeast Asia's overall malaria cases[3, 4]. Six countries accounted for more than half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (12%), Uganda (5%), and Côte d'Ivoire, Mozambique and Niger (4% each) [5]. Malaria is a vector-borne, tropical parasite disease occurring worldwide in 91 countries. The condition is triggered by the protozoal parasites of the genus Plasmodium [6, 7]. The most common species are Plasmodium falciparum and Plasmodium vivax, for which humans are the only mammalian hosts, and they are mostly responsible for the most significant public health burden[8, 9]. In addition to the few therapeutic options available, effective treatment of a protozoal disease is an enormous problem due to adverse effects, medication toxicity, resistance, long-term therapies, the susceptibilities of individual systems, and the parasite variability[10, 11].

Thiazole and thiosemicarbazone derivatives are widely described in the literature and present a wide range of biological activities[12]. There are various reasons that make thiazole scaffolds interesting
prototypes are their accessible chemical synthesis, low costs pertaining to reactivity, good yield reactions, and ability to generate a series of analogues, among others[13]. Thiazole motif has been widely explored by various researchers in the field of medicinal chemistry to combat various illnesses. In the normal functioning of the nervous systems, thiazole containing vitamin B1 (thiamine), for instance, helps to synthesize acetylcholine[14, 15]. Discovery of new medications for the protozoan parasitic related disorder is emergent. Several modifications on the thiazole ring at different positions led to the synthesis of several new compounds, with a broad spectrum of pharmacology activities. In this research, we have reported the synthesis of thiosemicarbazone and thiazole derivatives.

2. Experimental

2.1. Materials and methods

All the reagents and solvents were obtained from S.D. Fine chemicals, Sigma–Aldrich, CDH (central drug house), Loba chemie and E. Merck India Ltd. Melting point apparatus was used for the measurement of melting points of all derived compounds by using open glass capillaries and are uncorrected. Progress of the reactions was monitored by using TLC plates (silica gel G), hexane : ethyl acetate (3 : 2) and ethanol: chloroform (3:2) used as solvent systems. The spots were detected by spraying of iodine vapors or under UV-light. IR spectra were recorded on FT-IR, Agilent spectrophotometer by using KBr pellets in the range of 4000 – 400 cm$^{-1}$. The $^1$H NMR and $^{13}$C NMR spectra of the synthesized compounds were recorded on Bruker Top spin 3.2 instrument in solvent (CDCl$_3$); chemical shift ($\delta$) values reported in parts per million (ppm) using tetramethylsilane as internal reference. The splitting pattern of NMR spectra were abbreviated as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The mass spectra were recorded at $m/z$ values by using LC-MS spectrometer, Model Q-ToF Micro, Waters.

2.2. General procedure for the synthesis of thiosemicarbazone (1) (3i-3r)

A reaction involves 1 equivalent of substituted thiosemicarbazides were added with stirring to ethanolic warm solution of substituted aldehyde / ketone in presence of glacial acetic acid. The resulting mixture was refluxed with stirring at 80–90°C and the purity of the product as well as composition of reaction mixture were monitored by TLC (Thin layer chromatography). The reaction mixture were cooled down to room temperature and then recrystallized with suitable solvent.

2.2.1 [(E)-2-(4-methoxybenzylidene)-N-methylhydrazine-1-carbothiomide] (3i)
Pale yellow; Yield 68%; mp.205–206°C; Rf 0.64; IR (KBr) Vmax(cm⁻¹): 612 cm⁻¹ (C = S), 2215 cm⁻¹ (C = N), 1515 cm⁻¹ (C = C), 1256 cm⁻¹ (OCH₃), 3292 cm⁻¹ (N-H).

2.2.2 [(E)-2-(1-(4-hydroxyphenyl)ethylidene)hydrazine-1-carbothioimide] (3j)

White crystals; Yield 70%; mp.207–208°C; Rf 0.68; IR (KBr) Vmax(cm⁻¹): 605 cm⁻¹ (C = S), 2230 cm⁻¹ (C = N), 3400 cm⁻¹ (OH), 3107 cm⁻¹ (N-H).

2.2.3 [(E)-2-(4-hydroxy benzylidene)hydrazine-1-carbothioimide] (3k)

Pale yellow; Yield 81.07%; mp.198–199°C; Rf 0.70; IR (KBr) Vmax(cm⁻¹): 615 cm⁻¹ (C = S), 2215 cm⁻¹ (C = N), 3400 cm⁻¹ (OH), 3050 cm⁻¹ (C-H), 3290 cm⁻¹ (N-H).

2.2.4 [(E)-2-(4-hydroxy benzylidene)-N-methylhydrazine-1-carbothioimide] (3l)

Light yellow; Yield 70%; mp.208–209°C; Rf 0.68; IR (KBr) Vmax(cm⁻¹): 1530 cm⁻¹ (C = C), 2210 cm⁻¹ (C = N), 3445 cm⁻¹ (OH), 3150 cm⁻¹ (C-H), 3290 cm⁻¹ (N-H).

2.2.5 [(E)-2-(4-hydroxy benzylidene)-N-phenyl hydrazine-1-carbothioimide] (3m)

Pale yellow precipitates; Yield 51%; mp.205–206°C; Rf 0.67; IR (KBr) Vmax(cm⁻¹): 610 cm⁻¹ (C = S), 1532 cm⁻¹ (C = C), 2255 cm⁻¹ (C = N), 3400 cm⁻¹ (OH), 3200 cm⁻¹ (N-H).

2.2.6 [(E)-2-(1-(4-hydroxyphenyl ethylidene)-N-methyl hydrazineyl-1-carbothioimide] (3n)

Yellow; Yield 69%; mp.220–221°C; Rf 0.7; IR (KBr) Vmax(cm⁻¹): 625 cm⁻¹ (C = S), 2240 cm⁻¹ (C = N), 3295 cm⁻¹ (N-H), 3426 cm⁻¹ (OH).

2.2.7 [(E)-2-(1-(4-hydroxyphenyl)ethylidene-N-phenylhydrazine-1-carbothioimide] (3o)

Brown precipitates; Yield 69%; mp.210–211°C; Rf 0.63; IR (KBr) Vmax(cm⁻¹): 620 cm⁻¹ (C = S), 2262 cm⁻¹ (C = N), 3285 cm⁻¹ (N-H), 3400 cm⁻¹ (OH).

2.2.8 [(E)-2-(4-methoxybenzylidene)-N-phenylhydrazine-1-carbothioimide] (3p)
white crystals; Yield 70%; mp.195–196°C; Rf 0.60; IR (KBr) Vmax( cm⁻¹): 611 cm⁻¹ (C=S), 2262 cm⁻¹ (C=N), 3200 cm⁻¹ (N-H), 1260 cm⁻¹ (OCH₃).

2.2.9 [(E)-2-(1-4-bromophenyl)ethylidene]hydrazine-1-carbothioamide] (3q)

Yellow precipitates; Yield 58%; mp.251–253°C; Rf 0.7; IR (KBr) Vmax( cm⁻¹): 1532 cm⁻¹ (C=C), 2242 cm⁻¹ (C=N), 620 cm⁻¹ (C=O), 3290 cm⁻¹ (N-H), 1256 cm⁻¹ (OCH₃), 3150 cm⁻¹ (C-H), 3285 cm⁻¹ (N-H).

2.2.10 [(E)-2-(4-methoxy benzylidene)-N-methylhydrazine-3-carbothioamide] (3r)

Light yellow; Yield 70%; mp.197–199°C; Rf 0.65; IR (KBr) Vmax( cm⁻¹): 620 cm⁻¹ (C=S), 2210 cm⁻¹ (C=N), 1256 cm⁻¹ (OCH₃), 3150 cm⁻¹ (C-H), 3285 cm⁻¹ (N-H).

2.3. General procedure for the synthesis of thiazole derivatives (2) (Kp1-10)

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).

2.3.1. [(E)-2-(2-(4-methoxy benzylidene)hydrazineyl)-4-phenyl-2,3-dihydrothiazole] (Kp-1)

Cream precipitates; Yield 60%; mp. 180–181°C; Rf 0.5; IR (KBr) Vmax( cm⁻¹): 1121 cm⁻¹ (C-N), 1513 cm⁻¹ (C=C), 1258 cm⁻¹ (OCH₃), 3050 cm⁻¹ (C-H Aromatic), 3295 cm⁻¹ (N-H). ¹HNMR (300 MHZ CDCl₃) : 6 (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/z 323.41 (M⁺ +1).

2.3.2. [(E)-4-(1-2-(4-phenyl-2,3-dihydrothiazol-2yl)hydrazineylidene)ethyl]phenol] (Kp-2)

Yellow precipitates; Yield 75%; mp. 206–207°C; Rf 0.73. IR (KBr) Vmax( cm⁻¹): 1132 cm⁻¹ (C-N), 1513 cm⁻¹ (C=C), 3050 cm⁻¹ (C-H Aromatic stretching), 3127 cm⁻¹ (N-H stretch secondary amine), 3400 cm⁻¹ (OH stretching). ¹HNMR (300 MHZ CDCl₃) : 6 (ppm) 8.74 [s, 1H, CH]; 6.86–7.81 [m, Ar-H]; 7.02 [s, 1H, NH]; 6.54 [s, 1H, thiazole]; 5.50 [s, 1H, OH]; 2.24 [s, 1H, CH₃]. MS (EI) m/z 311.11 (M⁺ +1).
2.3.3. [(E)-4-((2-(4-phenyl-2,3-dihydrothiazol-2-yL)hydrazineylidene)methyl)phenol] (Kp-3)

Light brown precipitates; Yield 72%; 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).

2.3.4. [(E)-4-((2-(3-methyl-4-phenyl-2,3-dihydrothiazol-2-yL)hydrazineylidene)methyl)phenol] (Kp-4)

Dark yellow precipitates; Yield 69%; mp. 195–197°C, Rf 0.53. IR (KBr) Vmax(cm−1): 2735 cm−1(C-C Aromatic stretching), 3150 cm−1(C-H), 3447 cm−1(OH), 3500 cm−1(N-H).

2.3.5. [(E)-4-(1-(2–3,4-diphenyl-2,3-dihydrothiazol-2-yL)hydrazineylidene)ethyl)phenol] (Kp-5)

Light green precipitates; Yield 79%; 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).

2.3.6. [4-(1-(2-(3-methyl-4-phenyl-2,3-dihydrothiazol-2-yL)hydrazineylidene)ethyl)phenol] (Kp-6)

Yellow precipitates; Yield 85%; mp. 198–200°C; Rf 0.7. IR (KBr) Vmax(cm−1): 1606 cm−1(C = C), 1179 cm−1(C-N), 2940 cm−1(C-H), 3400 cm−1(OH stretch).

2.3.7. [(E)-4-(1-(2-(3,4-diphenyl-2,3-dihydrothiazol-2-yL)hydrazineylidene)ethyl)phenol] (Kp-7)

Blackish brown precipitates; Yield 77%; 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).

2.3.8. [(E)-2-(2-(4-methoxybenzylidene)hydrazineyl)-3,4-diphenyl-2,3-dihydrothiazole] (Kp-8)

Light grey; Yield 81.5%; mp. 211–213°C, Rf 0.45. IR (KBr) Vmax(cm−1): 1600 cm−1(C = C), 2826 cm−1(OCH3), 3147 cm−1(C-H), 3125 cm−1(N-H).
2.3.9. [(E)-2-(2-(1-(4-bromophenyl)ethylidene)hydrazineylidine)-3,4-diphenyl-2,3-dihydrothiazole] (Kp-9)

Orange; Yield 68%; mp 251–252°C; Rf 0.63

2.3.10. [(E)-N-(4-methoxy styryl)-3-methyl-4-phenyl-2,3-dihydrothiazole-2-amine] (Kp-10)

Yellow; Yield 87%; M.W 324.44; mp. 198–199°C; Rf 0.58

2.4. In vitro anti-malarial activity screening

The synthesized thiazole derivatives (Kp1-10) were evaluated for their anti-malarial activity and their results was expressed in terms of IC\textsubscript{50} µg/ml and are presented in Table 1. The in vitro anti-malarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann and co-workers 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].

2.7. Computational studies:

2.7.1. Pharmacokinetic (ADME) properties prediction

Physicochemical properties of the synthesized compounds(Kp1-10) were intended using QikProp module of Schrodinger software. Firstly, compounds were drawn in 2D using ChemDraw then further processed by using LigPrep module of Schrodinger's software as per standard protocols. All the prepared structures were introduced into QikProp module for the prediction of physicochemical properties. QikProp module
are used for the determination of pharmaceutically relevant properties such as QPLog octanol/water, QPlog S, PSA, acceptHB, donorHB[18–22].

### 2.7.2. Molecular docking

Molecular docking study was performed by GLIDE v3.8 (Schrodinger, LLC, New York) module for all thiazole derivatives. While (PDB code:5TBO) to predict the anti-malarial activity of newly tested compounds and preprocessed by using “protein preparation wizard” in Maestro module v10.3 (Schrodinger, LLC, New York). Some other steps like generate states and refinement have also been processed for addition of hydrogen atoms and disulfide bonds at the missing sites on protein molecule. The protein structure was changed to a single unit with the removal of water molecules and other unwanted subunits. After the optimization process, receptor grid generation was proceeded to the previously attached ligand site. The grid is associated with different sets of fields for determining shape and properties of the receptor. The ligand binds with protein residues by using specific force field, generate different poses. The best docked poses have been ranked on the basis of energy function combining empirical and force-field terms[23–26].

### 3. Results And Discussion

#### 3.1. Chemistry

In the present study, 10 thiosemicarbazone and thiazole derivatives have been synthesized which are outlined in scheme 1 and scheme 2. The starting material thiosemicarbazone (1) was prepared by the condensation reaction of substituted aldehyde/ketones with substituted thiosemicarbazides in the presence of ethanol/methanol with few drops of glacial acetic acid refluxing with stirring at 80–90°C for 8–9 hrs. The thiazole derivatives (2) was prepared by the reaction between thiosemicarbazone derivatives and phenacyl bromide refluxing in isopropyl alcohol for 5 hrs. General procedure for the synthesis of thiosemicarbazone and thiazole derivatives (Kp1-10) were achieved through the versatile and efficient synthetic route outlined in scheme 1 and scheme 2. Briefly, the synthesis of thiosemicarbazone and thiazole was achieved by using substituted aldehyde / ketone, in ethanol / methanol. Whereas, substituted thiosemicarbazides were added with stirring to ethanolic warm solution of substituted aldehyde / ketone in presence of glacial acetic acid. The resulting mixture was refluxed with stirring at 80–90°C and in second step thiosemicarbazones was further treated with phenacyl bromide and the purity of the product as well as composition of reaction mixture were monitored by TLC (Thin layer chromatography). The reaction mixture were cooled down to room temperature and then recrystallized with suitable solvent. The purity of the synthesized thiazoles was ascertained by TLC using Pre-coated Merk silica plate and Ethyl Acetate : Hexane (3 : 2) or chloroform : Hexane (3: 2) as the mobile phase. The spots were detected under UV at 245nm and R_f value was calculated. The structures assigned to the compounds were supported by the results of IR, ¹H NMR, ¹³C NMR and mass spectral data.
Structure of all the synthesized compounds was monitored by the FTIR, $^{1}$HNMR, $^{13}$CNMR, and Mass spectral analysis. Compound Kp-1 showed actual peak of OCH$_3$, C = N, N-H(stretching), C = C, C-H(aromatic), C-H(aliphatic) is at 1258 cm$^{-1}$, 1121 cm$^{-1}$, 3295 cm$^{-1}$, 1513 cm$^{-1}$. Compound Kp-2 showed actual peak of OH, C-H, C-N, N-H, C = C, C-H(aromatic), C-H(aliphatic) are at 3400 cm$^{-1}$, 3150 cm$^{-1}$, 1136 cm$^{-1}$, 1121 cm$^{-1}$, 3127 cm$^{-1}$, 1513 cm$^{-1}$. Compound Kp-3 showed actual peak of OH, C-H, C-N, N-H, C = C = C, C-H(aromatic), C-H(aliphatic) are at 3400 cm$^{-1}$, 3050 cm$^{-1}$, 1136 cm$^{-1}$, 1121 cm$^{-1}$, 3125 cm$^{-1}$, 1513 cm$^{-1}$. Compound Kp-4 showed actual peak of OH, C-H, C-C are at 3447 cm$^{-1}$, 3150 cm$^{-1}$, 2735 cm$^{-1}$. Compound Kp-5 showed actual peak of C = N, C-H, C-N, O-H are at 2228 cm$^{-1}$, 3108 cm$^{-1}$, 1226 cm$^{-1}$, 3400 cm$^{-1}$. Compound Kp-6 showed actual peak of C = C, C-N, O-H, are at 1606 cm$^{-1}$, 1179 cm$^{-1}$, 3326 cm$^{-1}$, 2940 cm$^{-1}$. Kp-7 showed actual peak of O-H, C = C, C-N, is at 3400-3650 cm$^{-1}$, 1600&1475 cm$^{-1}$, 1030–1230 cm$^{-1}$, but their expected values are at 3434 cm$^{-1}$, 1599 cm$^{-1}$, 1230 cm$^{-1}$. Compound Kp-8 actual peak of O-CH$_3$, C = C, C-N, are at 1236 cm$^{-1}$, 1600 cm$^{-1}$, 3147 cm$^{-1}$, 3334 cm$^{-1}$. Compound Kp-1 showed $^{1}$HNMR chemical shift peak at δ 6.25–7.38 due to aromatic proton. Chemical shift peak at δ 4.02 due to OCH$_3$ group attached to aromatic ring. Compound Kp-2 showed multiplet at δ 6.86–7.81 due to aromatic proton. Compound with OH group attached to aromatic ring showed chemical shift peak at δ 5.50 and chemical shift peak at δ 7.02 due to aliphatic NH. The mass spectra of compound Kp-1 [(E)-2-(2-(4-methoxybenzylidene)hydrazinyl)-4-phenyl-2,3-dihydrothiazole] showed peak at m/z 323.41. Compound Kp-2 [(E)-4-(1-2-(4-phenyl-2,3-dihydrothiazol-2yL)hydrazinylidene)ethyl]phenol] showed peak at m/z 311.11.

### 3.2. In vitro antimalarial activity screening

The antimalarial activity of the synthesized thiazole derivatives (Kp 1–10) was assessed against human pathogenic malarial strain viz. Plasmodium falciparum while quinine was taken as the standard drug. Activity profile of all the compounds screened for antimalarial activity is represented in Table 1. Results antimalarial evaluation revealed that most of the tested compounds exhibited remarkable inhibitory activity against the tested plasmodium strain. Among them, compound Kp-9 was found to be most promising which exhibited strongest inhibitory activity against P. falciparum with an IC$_{50}$ value of 0.29µg/mL which was higher than the reference drug quinine (1.26µg/mL). Compound 10 also presented excellent inhibition of P. falciparum with IC$_{50}$ values of 0.59 respectively. Other tested derivatives such as Kp-1, Kp-3 and Kp-4 also exhibited significant antimalarial activity with IC$_{50}$ values in the range of 0.65, 0.90, 0.85µg/mL. Compound Kp-2 was the least potent candidate of the series with IC$_{50}$ value of 5.52 µg/mL.

The structure-activity relationship of the various titled compounds (Kp-1-10) screened for antimalarial activity has been analysed using quinine as the reference drug. Results of in vitro evaluation data indicated that inhibitory activity of the various tested compounds against P. falciparum depends upon the nature and type of substituents introduced at the 5th position of the thiazole core. Substitution with methyl group or bulky groups in compound Kp-2, Kp-6 at R decrease the anti-malarial activity whereas substitution with hydrogen group at same position responsible for increase in activity as shown by compound Kp-2, Kp-6 and Kp-7. Substitution with electron withdrawing group at R$_1$ increases anti-
malarial activity as shown in compound Kp-9. Substitution at R₂ with hydrophobic group responsible for decrease in activity as shown in compound Kp-5, Kp-7 and Kp-8. Compounds having hydrogen or hydroxyl group at R and R₂ position shown in compound Kp-1 showed promising anti-malarial activity. The present study highlighted that most of the synthesized thiazole derivatives possessed strong inhibitory potential against human pathogenic malarial strain P. falciparum.

3.3. Computational studies

3.3.1. Pharmacokinetic (ADME) properties prediction

The ADME properties of all previously synthesized compounds have been calculated in comparison with active molecules (Table 2). It has been observed that though all the ADME properties of compounds are well within the acceptable range. Here, proposed compounds showed high percentages of human oral absorption and partition coefficient. Furthermore, synthesized derivatives also showed good cell permeability. From all these results it has been observed that these analogs by making no changes in the core fused scaffold and isosteric/bioisosteric and knowledge based side chains and fragment attachment came up with very potential lead molecules with favorable drug-like profile which can emerge as a potential drug molecule in further development. Qikprop predictions suggested that thiazole derivatives have optimum parameters for anti-malarial activity and can be considered as a lead molecule for further modifications.
### Table 2
Molecular descriptors of thiazole derivatives.

| Compound code | R    | R<sub>1</sub> | R<sub>2</sub> | Yield | Melting point | Mean IC<sub>50</sub>(µg/ml) |
|---------------|------|---------------|---------------|-------|---------------|----------------------------|
| Kp-1          | H    | OCH<sub>3</sub> | H             | 60%   | 180–181 °C    | 0.65                       |
| Kp-2          | CH<sub>3</sub> | OH | H             | 75%   | 206–207 °C    | 5.52                       |
| Kp-3          | H    | OH            | H             | 82%   | 210–212 °C    | 0.90                       |
| Kp-4          | H    | OH            | CH<sub>3</sub> | 69%   | 195–197 °C    | 0.85                       |
| Kp-5          | H    | OH            | Ph            | 73%   | 200–202 °C    | 2.80                       |
| Kp-6          | CH<sub>3</sub> | OH | CH<sub>3</sub> | 85%   | 198–200 °C    | 3.05                       |
| Kp-7          | CH<sub>3</sub> | OH | Ph            | 78%   | 198–199 °C    | 4.80                       |
| Kp-8          | H    | OCH<sub>3</sub> | Ph           | 81.5% | 211–213 °C    | 3.19                       |
| Kp-9          | CH<sub>3</sub> | Br | H             | 68%   | 251–252 °C    | 0.29                       |
| Kp-10         | H    | OCH<sub>3</sub> | CH<sub>3</sub> | 87%   | 198–199 °C    | 0.59                       |

| Compounds     | M.W  | PSA   | donorHB | acceptHB | QPlogS   | QPLogPo/w |
|---------------|------|-------|---------|----------|----------|-----------|
| Kp-1          | 311.401 | 44.243 | 1       | 4.75     | -5.217   | 4.188     |
| Kp-2          | 311.401 | 56.068 | 2       | 4.25     | -4.92    | 3.742     |
| Kp-3          | 297.374 | 58.598 | 2       | 4.75     | -4.694   | 3.337     |
| Kp-4          | 311.40  | 56.734 | 2       | 4.25     | -4.571   | 3.673     |
| Kp-5          | 373.12  | 49.141 | 2       | 3.75     | -6.821   | 6.015     |
| Kp-6          | 325.42  | 49.122 | 2       | 3.75     | -5.451   | 4.544     |
| Kp-7          | 387.5   | 49.137 | 2       | 3.75     | -6.707   | 5.99      |
| Kp-8          | 387.5   | 41.902 | 1       | 4.25     | -6.93    | 6.127     |
| Kp-9          | 450.395 | 24.859 | 1       | 3        | -7.843   | 7.266     |
| Kp-10         | 324.44  | 43.411 | 0       | 3.25     | -5.495   | 5.395     |

### 3.3.2. Molecular docking

Molecular modeling studies were performed on Glide v5.8 (Schrodinger, LLC, New York, NY) to investigate the potential interactions between target compound and (PDB code: 5TBO). All synthesized compounds (Kp1-10) were docked for studying the essential interactions of compounds with protein to produce antimalarial activity. All the thiazole derivatives were docked (PDB 5TBO) for studying the binding mode of
compounds for anti-malarial activity. The potent thiazole derivatives Kp-9, showed highest docking score such as (-9.519) Therefore, compounds Kp-1, Kp-3, Kp-4 and Kp-10 also showed highest docking Score(-8.764, -8.406, -9.062, -9.435 respectively) and critical interactions with VAL532, ILE237, LEU531, HIE185, TYR528, ASN274, ARG265 whereas yellow colour shows H-bond interactions, Purple colour shows Halogen bond, green colour shows pi-cation, green colous shows hydrophobic interactions, blue colour shows polar, green dotted lines shows Pi-Pi stacking.

4. Conclusion

In the present study, a series of thiosemicarbazone and thiazole derivatives have been synthesized, characterized, and screened against potential anti-malarial activity. The anti-malarial activity of the synthesized thiazole derivatives (Kp1-10) was assessed against human pathogenic malarial strain viz. Plasmodium falciparum while quinine was taken as the standard drug. Compound Kp-9 was found to be most promising which exhibited strongest inhibitory activity against Pfalciparum with an IC$_{50}$ value of 0.29µg/mL which was higher than the reference drug quinine (1.26µg/mL). The SAR study revealed that the substitution with electron withdrawing group at phenyl increases anti-malarial activity as shown in compound Kp-9. The result of molecular docking studies showed that compounds Kp-9, Kp-1, Kp-3, Kp-4 has crystal alignment as crystal ligand of protein(PDB code: 5TBO) and compound Kp-9 showed highest docking score such as (-9.519) Therefore, compounds Kp-1, Kp-3, Kp-4 and 10also showed highest docking Score(-8.764, -8.406, -9.062, -9.435 respectively) and critical interactions with VAL532, ILE237, LEU531, HIE185, TYR528, ASN274, ARG265. 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 Kp-9 may be considered for the development of newer anti-malarial agents.

Declarations

Conflict of Interest:

The authors declare no conflict of interest.

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**Table**

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.
Figures

Chemical structures of thiosemicarbazone derivatives

Figure 1

Chemical structures of thiosemicarbazone derivatives
Figure 2

Chemical structures of thiazole derivatives
Figure 3

Docking results showing interaction of compound Kp-9 with amino acid residues GLN 93, ASP 127 and TYR 264 of plasmodium falciparum (PDB Code: 5TBO).
Figure 4

Docking results showing interaction of compound Kp-1 with amino acid residues GLU 192, ASP 127 and LEU 189 of plasmodium falciparum (PDB Code: 5TBO).
Figure 5

Docking results showing interaction of compound Kp-3 with amino acid residues PHE 227 of Plasmodium falciparum (PDB Code: 5TBO).
Figure 6

Docking results showing interaction of compound Kp-3 with amino acid HIE 185 residues of plasmodium falciparum (PDB Code: 5TBO).
Figure 7

Docking results showing interaction of compound Kp-3 with amino acid PHE 227 residues of plasmodium falciparum (PDB Code: 5TBO).
Synthetic scheme 1:

Scheme 1. Reagents: (a) EtOH, Glacial acetic acid (c) substituted thiosemicarbazides (d) substituted aldehyde/ketone

| Compound code | R   | R₁   | R₂   |
|---------------|-----|------|------|
| 3i.           | H   | OCH₃ | H    |
| 3j.           | CH₃ | OH   | H    |
| 3k.           | H   | OH   | H    |
| 3l.           | H   | OH   | CH₃  |
| 3m.           | H   | OH   | Ph   |
| 3n.           | CH₃ | OH   | CH₃  |
| 3o.           | CH₃ | OH   | Ph   |
| 3p.           | H   | OCH₃ | Ph   |
| 3q.           | CH₃ | Br   | H    |
| 3r.           | H   | OCH₃ | CH₃  |

Figure 8

Scheme 1. Reagents: (a) EtOH, Glacial acetic acid (c) substituted thiosemicarbazides (d) substituted aldehyde/ketone
Synthetic scheme 2:

\[ \text{Thiosemicarbazone derivatives} \quad (3a-r) \]
\[ \text{R} = \text{H, CH}_3 \]
\[ \text{R}^1 = \text{Substituted Aldehydes, Substituted Ketones} \]
\[ \text{R}^2 = \text{H, Ph, Me} \]

Scheme 2: Synthesis of thiazole derivatives

| Compound code | R  | R1   | R2   |
|---------------|----|------|------|
| Kp-1.         | H  | OCH$_3$ | H    |
| Kp-2.         | CH$_3$ | OH | H    |
| Kp-3.         | H  | OH   | H    |
| Kp-4.         | H  | OH   | CH$_3$ |
| Kp-5.         | H  | OH   | Ph   |
| Kp-6.         | CH$_3$ | OH | CH$_3$ |
| Kp-7.         | CH$_3$ | OH | Ph   |
| Kp-8.         | H  | OCH$_3$ | Ph   |
| Kp-9.         | CH$_3$ | Br | H    |
| Kp-10.        | H  | OCH$_3$ | CH$_3$ |

Figure 9

Scheme 2. Reagents: (a) isopropyl alcocol (c) substituted thiosemicarbazones (d) phenacyl bromide

Supplementary Files

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- Table1.docx