

In silico and in vivo anti-malarial investigation on [(5-nitroheteroaryl-2-yl) methylidene] hydrazinyl hetroaryl derivatives

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Abstract

Background Today, the resistance to *Plasmodium falciparum* against common anti-malarial drugs has attracted the attention towards the alternative and effective drugs. Synthetic derivatives of [(5-nitroieroaryl-2-yl) methylidene] hydrazineyl heteroaryl showed in vitro anti-plasmodial activity. The aim of this study was to evaluate the molecular binding and antiplasmodial activity of in vivo synthetic compounds.

Methods: The molecular docking was used to study the binding of compounds to heme and *Plasmodium falciparum* lactate dehydrogenase (PfLDH). Acute toxicity of the synthetic compounds was evaluated based on modified up & down method. Anti-plasmodial activity of the compounds was conducted by two standard methods of Peter and Rane's tests via chloroquine-sensitive *Plasmodium berghei*. Also, the toxicity of mice's internal organs was evaluated on day 7 in addition to the histopathology of their liver.

Results The docking studies showed that active site of PfLDH had at least four common residues including Ala98, Ile54, Gly29 and Tyr97 to bind the compounds with the affinity ranging from -8.0 to -8.4 Kcal/mol. The mode of binding of ligands to heme revealed effective binding affinity ranging from -5.1 to -5.5 Kcal/mol. Compound 2 showed the highest % suppression of parasitemia (99.09%) at the dose of 125mg/kg/day in Peter's tests. Compound 3 with 79.42% suppression was the best compounds in Rane's test at the lowest dose (31.25 mg/kg/day). The histopathology of the mice's livers did not reveal the focal necrosis of hepatocytes in the studied compounds.

Conclusions The docking studies verified Pf LDH inhibition and the inhibitory effect on the hemozoin formation for the studied compounds. Accordingly, some compounds may provide new achievements for the development of antimalarial drugs without liver toxicity, although further studies are required to optimize their anti-plasmodial activity.

Background

Malaria is one of the most important infectious diseases that threaten half of the world's population [1]. The world malaria report 2018 estimated that there were 219 million cases of malaria in 2017 causing 435,000 global deaths. The 10 highest malaria-stricken African countries had an estimated 3.5 million more malaria cases in 2017 than the previous year [1]. There was no impressive achievement in reducing malaria cases during 2015 - 2017. However, there is a big challenge to thoroughly eliminate malaria by 2030 [2].

Quinoline (chloroquine (CQ), Fig. 1) and artemisinin (ART, Fig. 1) derivatives are the two main classes of antimalarial drugs [3]. However, repetitive and inappropriate use of CQ caused drug resistance in *Plasmodium* parasite. The decreased susceptibility of parasites to artemisinin in the Greater Mekong Sub-region (GMS): Laos, Myanmar, Cambodia, Thailand and Southern Vietnam may extend to other endemic areas [4, 5]. In fact, the epidemiological evidence predicts "tsunami" of artemisinin resistance in the world, called "super malaria". In this situation, artemisinin-based combination therapies (ACTs) is the

first-line treatment in areas with artemisinin resistance which has unfortunately showed ACTs' failure in some region, due to both decreased susceptibility to artemisinin and partner drug resistance [6]. Since no effective vaccine against malaria has ever been produced, pharmacotherapy can be of great interest in the current situation. Several research groups worked on the development of novel antimalarial drug pipelines with some chemical modification on the current drugs [7, 8]. Moreover, natural products are noteworthy as new sources of antiplasmodial [9, 10]. In fact, new, effective, low-cost, safe, and affordable alternative antimalarial agents are urgently required [11].

Given the importance of the subject, we designed and synthesized new analogs of quinoline antimalarial drugs by integrating the important biological rings of quinoline and quinazoline with the active fragment of 5-nitroeroaryl methylidene hydrazine, inspired by antimicrobial drugs, nitrofurantoin and nifurtimox (Fig 1). Nitrofurantoin is an antibiotic used to treat bladder infections and prophylaxis of infection [12]. *Nifurtimox* is effective in the treatment of [sleeping sickness](#) and [Chagas disease](#) [13]. In last research, plasmodium falciparum lactate dehydrogenase assay (PfLDH assay) showed that the synthetic compounds were effective against CQ-sensitive (3D7) and CQ-resistant (K1) Plasmodium falciparum (Pf) strains [14]. Also, hemozoin formation in 3D7 and (K1) *P. falciparum* strains with synthetic compounds was measured via β -hematin assay [14]. The results of β -hematin assay were equal to PfLDH assay. In the present study, the binding of the synthetic compounds to heme and PfLDH were studied by the molecular docking. Also, given the importance of in vivo tests, the anti-plasmodial activity of the compounds was assessed by Peter's and Rané's tests in mice inoculated with *Plasmodium berghei* (ANKA strain). The histological changes in the liver mouse malaria model were evaluated at day 7 after the treatment.

Material And Methods

Materials and chemical reagents were purchased from Sigma-Aldrich Company (USA). Compounds **1** to **4** were prepared according to our previous research [14]. The binding of ligands to heme and PfLDH were studied based on the molecular docking via AutoDock tool. The female BALB/C mice were purchased from Pasteur Institute of Iran. Chloroquine sensitive *Plasmodium berghei* (ANKA) strain was obtained from department of Medical Entomology, School of Public Health, Tehran University, Tehran, Iran.

Receptor structure preparation and generation of grid box for docking studies

Molecular docking is a worthwhile method to explore protein and ligand interactions at the molecular level [15]. The method was used to study the binding of ligands to heme and plasmodium falciparum L-lactate dehydrogenase (pLDH). The 3D structure of the L-lactate dehydrogenase and heme were taken from the Protein Data Bank (PDB ID 1LDG and 3P5Q, respectively) for molecular docking experiment. To this end, the receiver structure was prepared using the AutoDock toolkit. The missing atoms were added, residues were assigned, and the AutoGrid parameter file was adjusted via AutoDock tools [16]. The L-lactate dehydrogenase active site was selected as the ligand binding site [17]. The size of the docking

grid for L-lactate dehydrogenase and heme were X= 32 Å Y=30 Å and Z=32 Å and X= 14 Å , Y=10 Å and Z=14 Å , respectively. The grid spacing was adjusted on 1 Å.

Ligand Molecule Preparation

The ligand molecules, drawn by Marvin 19.10 (<http://www.chemaxon.com>) were subsequently optimized via HyperChem 8.0 software [18].

Molecular Docking Study

The ligand molecules were docked in the selected binding pocket via Smina AutoDock. Smina is a version of AutoDock Vina, focusing on the improvement of scoring and energy minimization [19]. Then, the crystal structure of receptors in complex with ligands was analyzed via LigPlot⁺ software [20].

In vivo anti-malarial assay

Acute toxicity of the compounds in mice

Before in vivo study, the toxicity of the compounds was assessed by Dixon's up and down method in BALB/c mice with some modification [21]. In the first phase, 500 mg/kg of compounds administered intraperitoneally to two mice and observed for any signs of toxicity and mortality at 24 h. *When a mouse* died, the concentration was reduced to half, the test was repeated, and the alive mice were monitored for toxicity and mortality for 10 days. In the second phase, test was repeated with 5 mice, and if mice survived, the highest non-toxic dose would be determined for Rane and Peter's tests (125 mg/kg).

Parasite inoculation

At first, two female BALB/C mice were infected intraperitoneally from frozen stock of CQ-sensitive *P. berghei* (ANKA) strain. Then, four and six mice were infected by continuous intraperitoneal passage. Next, the blood was diluted with PBS and finally the experimental mice were infected with an inoculum of 1.5×10^8 of parasitized erythrocytes intraperitoneally.

Schizonticidal effect in early infection (Peter's test)

The suppressive test was conducted in accordance with Peter's method [8, 22]. The experimental mice were maintained under the standard conditions based on the international guidelines for ten days [23]. Seventy-five female BALB/C mice (weight 16-20 g) were grouped into fifteen groups. They were inoculated intra-peritoneally (i.p.) with 1.5×10^7 infected erythrocytes of CQ-sensitive *P. berghei* in a saline (200 μ l) on the first day (D0) of the test.

The test compounds were solubilized in a mixture of DMSO, sesame oil, and ethanol (a ratio of 1:2:0.05) pre-diluted in sesame oil for preparation of different dose (31.25, 62.5 and 125 mg/kg). The treatments began within 3 h post-inoculation of mice with the parasite (D0) and proceeded intraperitoneally for five days (D4).

Tail blood smear was taken, stained with 10% Giemsa in phosphate buffer (pH 7.2) on the fifth day (D4). The parasitaemia level was determined by counting the parasitized red blood cells on at least 2,000 red blood cells by microscope at 100 \times . The %suppression of parasitaemia was calculated by comparing the %parasitaemia between test mice and infected controls. The chloroquine diphosphate (25 mg/kg), oil and DMSO (12.5%) were applied as positive and negative controls, respectively. For all the groups, mortality was monitored daily and mean survival time was recorded to evaluate the efficacy of anti-malarial activity of the compounds. During the treatment, the body weight of each mouse was measured before the infection (D0) and after treatment (D4). Also, the internal organs (spleen, liver, and kidney) were evaluated on the seventh day of treatment after the dissection of the mice.

Schizontocidal activity in established infection (Rane's test)

The curative test was conducted according to the method of Ryley and Peters (Rane's test) [24]. The animal housing, infecting, and dosing were done similar to Peter's test. Nevertheless, the treatment began within 72 h post-inoculation of mice with the parasite (D2) to allow parasitemia to establish and then continue for four days (D7) intraperitoneally. The tail blood smears were taken, stained with 10% Giemsa in phosphate buffer (pH 7.2) on the 8th day (D7). The parasitaemia level and the %suppression of parasitaemia, mortality, mean survival time, and body weight were determined and recorded in line with Peter's test.

Histopathological study of liver tissue

Histopathological studies were conducted based on the standard method [25]. After the dissection on seventh day after treatment, mice's livers were fixed in 10% formalin solution. Then, the middle lobe of livers was severed and *molds* of fresh *paraffin* were made of tissue with a thickness of 5 μ m. The sections were stained with hematoxylin–eosin (H&E) and analyzed by light microscope. The slides were examined under 40 \times objective lens and image saved as JPEG files. The sections were scored from 0–4

for sinusoidal congestion, vacuolization of hepatocyte cytoplasm, and parenchymal single cell necrosis as described by Suzuki et al. [26].

Statistical analysis

The data were analyzed using SPSS (IBM SPSS Statistics 22). Then one-way ANOVA was used to test statistical differences for three doses within a group, followed by Tukey's test for multiple comparisons. *P*-values < 0.05 were considered significant in all tests.

Result

In silico investigation of molecular interactions

The MarvinSketch was used to draw ligands molecule and optimize with HyperChem software. Shown in Fig. 1, the ligands were docked at heme and the active site of the L-lactate dehydrogenase in AutoDock Smina to generate the best possible conformations with the lowest binding energies. The molecular docking results demonstrated that L-lactate dehydrogenase complexes with lig1, lig 2, lig 3 and lig 4 with the range of binding affinity as -8.2 to -7.4, -8.0 to -7.0, -8.4 to -7.4 and, -8.2 to -6.8 kcal/mol, respectively. These values for heme complexes were -5.4 to -4.7, -5.1 to -4.6, -5.5 to -4.9 and -5.2 to -4.8, respectively. The results of the best binding affinities are tabulated in Table 1. The binding mode interactions were analyzed using Ligplot software. The significant L-lactate dehydrogenase residues involved in the interaction with ligands were demonstrated in Fig. 2. Moreover, the molecular docking results showed that the binding pocket of ligands had at least four common residues, including Ala98, Ile54, Gly29 and Tyr97. The illustration of binding modes interactions of complexes were shown in Figure S1, [supplementary material].

Table 1 Evaluation of best binding affinity of L-lactate dehydrogenase and heme with the studied ligands

Complexes	Best binding affinity to PfLDH(Kcal/mol)	Complexes	Best binding affinity to Heme (Kcal/mol)
PfLDH-lig1	-8.2	Heme-lig1	-5.4
PfLDH - lig2	-8.0	Heme-lig2	-5.1
PfLDH - lig3	-8.4	Heme-lig3	-5.5
PfLDH - lig4	-8.2	Heme-lig4	-5.2

To study the mode of binding of ligands to heme, the best docked complexes were subjected to Ligplot⁺, the analysis that allows the identification of the ligand-receptor contacts. The involved atoms in ligand binding were represented in Fig. 3.

In vivo acute toxicity of the synthetic compounds

The examined mice at the dose of 125 mg/kg were normal showing no behavioral and physical changes after 10 days. Three test doses of the synthetic compounds were selected based on the highest non-toxic dose (125mg/kg) for the anti-plasmodium activity evaluation in mice.

Evaluation of schizonticidal activity in early infection for the compounds (1- 4)

The mice treated with 125 mg/kg dose showed 61.69, 99.09, 66.50 and 69.39% chemosuppressive antimalarial activity for groups **1a- 4a**, respectively. The group **3c** showed the most activity at lower dose (78.74%) (Table 2, Fig. 4A). The control groups died two weeks after the infection. The body weight on the fifth day (D4) decreased compared with first day (D0) (Table 2, Fig. 4B).

Table 2 Evaluation of average % parasitemia and % suppression of parasitemia in Peter's test

P-value	Average % parasitemia ± SD (Day 8)	Suppression of % (parasitemia (Day 4	Average % parasitemia ± SD (Day (4	Dose (mg/kg)	Comp	Groups
-	10.68±1.11	-	5.33±2.12	-	-	Oil
-	13/16±1.47	-	7.41±1.65	-	-	DMSO
-	0	100	0	25	-	CQ
0.05 >	3.20±1.46	61.69	1.56 2.44±	125	1a	1
	6.847±0.45	47.10	3.37±2.39	62.5	1b	
	8.887±2.25	8.01	5.86±1.06	31.25	1c	
0.05 >	2.75±0.84	99.09	0.058±0.17	125	2a	2
	4.85±1.30	54.16	2.92±2.79	62.5	2b	
	9.30±2.75	5.49	6.02±1.79	31.25	2c	
<	2.547±0.42	66.50	2.134±1.75	125	3a	3
0.05	8.182±0.97	42.26	3.678±0.76	62.5	3b	
	7.255±1.07	78.74	1.354±1.86	31.25	3c	
0.05 >	4.262±2.17	69.39	1.95±0.44	125	4a	4
	7.54±2.52	43.01	3.63±2.51	62.5	4b	
	9.957±2.27	20.41	5.07±2.54	31.25	4c	

The *in vivo* activities were evaluated against *Plasmodium berghei*.

SD: Standard Deviation

A mild enlargement of the liver in the treated groups was observed after the dissection of the internal organs (Fig. 4C) compared with the control groups especially for groups **3a-c** and **4a**. The quantitative evaluation of the treated mice's spleen showed a considerable increase comparing to the CQ except groups **2a-b** and **4b-c** (Fig. 4C). The treatment groups survived longer than the control groups except group **4c** as shown in Fig. 4D. There was no significant change in the kidneys of treated groups.

Evaluation of schizontocidal activity in established infection for the compounds (1- 4)

The mice treated with 125 mg/kg dose showed 53.75, 71.07, 61.53 and 50.08% chemo-suppressive antimalarial activity for groups **1a- 4a**, respectively. The group **3c** similar to peter's test showed the most activity at lower dose (79.42%) (Table 3 and Fig. 5A). On the day 8, the body weight (D7) decreased (Table 3, Fig. 5B). The mice treated with the synthetic compounds significantly survived longer than the control groups except groups **1b, 3a** and **4a** as shown in Table 3, Fig. 5C.

Table 3 Evaluation of average % parasitemia and % suppression of parasitemia in Rane's test

P-value	CLogP	Suppression of parasitemia %	Average % parasitemia ± SD	Dose (mg/kg)	Comp	Groups
		((Day 7	((Day 7			
-	-	-	5.33±2.17	-	-	Oil
-	-	-	5.46±0.94	-	-	DMSO
-	5.06	100	0	25	-	CQ
0.05 <	3.93	53.75	1.67 2.49±	125	1a	1
		52.72	2.55±1.30	62.5	1b	
		11.11	4.79±1.22	31.25	1c	
0.05 <	4.46	71.07	1.56±1.53	125	2a	2
		57.17	2.31±1.27	62.5	2b	
		53.05	2.53±0.95	31.25	2c	
>	2.50	53.61	2.50±1.08	125	3a	3
		49.56	2.72±2.10	62.5	3b	
		79.42	1.11±0.81	31.25	3c	
0.05 <	3.03	50.08	2.69±1.02	125	4a	4
		36.25	3.44±1.95	62.5	4b	
		28.87	3.84±0.58	31.25	4c	

The *in vivo* activities were evaluated against *Plasmodium berghei*.

SD: Standard Deviation

Histopathological study of mice livers

The histopathological study of the livers of the treated mice with the synthetic compounds after 7 days of treatment was evaluated in higher studied dose (125 mg/kg) for Peter's test. The liver of the mice did not show any damage such as vacuolization, minimal congestion, and single-cell necrosis. While, the mice which received CQ, showed minimal sinusoidal animals congestion, vacuolization and single cell necrosis (Table 4, Fig. 6).

Table 4 Histopathological evaluations of the liver

Comp	Congestion	Vacuolization	Necrosis	Score
Oil	None	None	None	0
CQ	Minimal	Minimal	Single cell necrosis	1
1	None	None	None	0
2	None	None	None	0
3	None	None	None	0
4	None	None	None	0

Statistical analysis

The variance between the groups was compared to within-group in Peter's test and showing a significant difference between the three studied dose groups ($P < 0.05$). The statistical analysis of Rane's test showed no significant difference between three studied dose groups ($P > 0.05$). Also, the *Tukey multiple comparisons* for groups 1 and 2 showed no significant difference between the means ($P > 0.05$) which could confirm the results of variance analysis.

Discussion

The anti-plasmodial activity of the synthetic compounds (1–4) was evaluated against *P. berghei* with suppressive and curative tests of Peter and Rane, respectively [22, 24]. In previous work, the compounds were tested against *P. falciparum*. The best result of pLDH method was related to compound 1 with (nitrofuranyl) methylene) hydrazine side chain and 7-chloro quinoline ring (IC₅₀ values of 0.52 and 1.00 mg/ml against 3D7 and K1 strains, respectively) [14]. Its thiophen analog (2) showed IC₅₀ values of 0.84 and 1.04 mg/ml against 3D7 and K1 strains, respectively. The compounds 1 and 2 were more active than the reference drug (CQ) against K1 strain. The compounds 3 and 4 with quinazolin ring displayed weaker results. The mode of action of CQ (Fig. 1) was related to the interference with the detoxification of free heme in the lysosomal digestive vacuole of the parasite which was essential for its continuous growth and proliferation. Therefore, based on the structural similarity of the synthetic compounds with 4-aminoquinoline anti-malarials, their mode of action was evaluated by hemozoin formation assays in previous research. The compounds (1–2) with the appropriate anti-plasmodial activity showed higher inhibitory activity against hemozoin synthesis.

In this research, the compound was docked at the active site of the L-lactate dehydrogenase and heme in AutoDock Smina [19]. The best possible conformations with the lowest binding energies were identified (Table 1). The silico studies presented an effective and close binding energy toward the target proteins PLDH and Heme ranging from - 8.0 to -8.4 and 5.1–5.5 kcal/mol, respectively. The compound 2 showed five hydrogen binding interactions with active site amino acids Ile31, Thr97, Gly32, Lys51, Asp53 and Thr85 having energy - 8.0 kcal/mol exhibiting promising interaction on active site of PfLDH ((Table 1, Fig. 2, S1). Based on the binding mode of ligands to heme, 1 and 3 have the highest binding affinity. However, the differences were very insignificant.

The compounds studied at the concentrations of 125, 62.5 and 31.25 mg/kg, respectively for in vivo tests against inoculated mice with *P. berghei*. The oil and dimethyl sulfoxide control groups showed higher parasitemia than the studied compounds (Tables 2–3 and Figs. 4–5).

Compound 2 in Peter's test showed the highest % suppression of parasitemia (99.09%) on the fifth day after treatment (Table 2, Fig. 4). Also, compound 2 was the most active compound in Rane's test with 71.07% suppression of parasitemia on the 8th day after treatment (Table 3, Fig. 5). This was notable that the compound had the highest lipophilicity among the four compounds studied and easily could cross the biological barriers (Table 3; ClogP = 4.46), while compound 1 showed the best result based on in vitro test (CLogP = 3.93). The comparison between the results of in vitro and in vivo tests verified the importance of in vivo test.

The best %growth inhibition (78.74%) for compound 3 was observed in the lowest dose (31.25 mg/kg) five days after the treatment (Table 2). Whereas, the highest concentration (125 mg/kg) showed the best result on day 9 after treatment with 2.55% average Parasitemia (Table 2). Compound 3 had a slower absorption at the highest concentration, but over time it could slowly accumulate in the blood with the high concentration and kill Parasites. It was confirmed in the toxicity test on 7 day, after the dissection of mice, the oily layer were observed in the abdominal area in the highest dose which confirms the slow absorption of the compound 3 at this concentration. Probably at lower concentration, the compound had a high initial uptake rate or is capable to eliminate the parasite in the blood before the parasite entered the red blood cell, and after a few days due to its low concentration, the parasites would grow easily.

The toxicity of target compounds on the seventh day after treatment was done and weight loss and discoloration of the internal organs could be a reason for the toxicity of these compounds (Fig. 4). However, it may be affected by the lack of complete elimination of the parasites during the period of treatment.

The anti-plasmodium effect was also evaluated by Rane's test. Compound 2 at the highest concentration (125 mg/kg) and compound 3 at the lowest concentration (31.25 mg/kg) showed the best % inhibition of parasite growth with 71.07 and 79.42%, respectively on the eighth day after treatment (Table 3). This test also emphasized that compound 3 had the best result at the beginning of treatment with the lowest concentration. Compound 2 (the best compound based on the Peter's test) showed 71% of the growth inhibition in the Rane's test, where the treatment begins three days after the inoculation of parasite and the parasite finds an opportunity to grow (Tables 2 and 3).

Based on the histopathological study, minimal congestion, vacuolization, and single cell necrosis were observed in the liver of CQ treated mice; however, they were not observed in the other groups which confirmed the fact that the target compounds were safe for the liver [25]. This requires more investigations in the future.

Although, %parasitemia decreased less than CQ, the need for a new, safe, well-tolerated and low-cost alternative drug is seriously felt due to the spread of CQ resistance in many areas of the world. It seems that these compounds have appropriate potential to replace quinoline antimalarial drugs for simple synthesis, proper yield, and suitable anti-plasmodial activity.

Conclusion

In this study, the results of in vivo tests and its comparison with in vitro tests verified that these compounds are effective on the blood stage of Plasmodium parasite. The results of docking studies corroborated that hydrogen bonding and several hydrophobic interactions between the ligands and the binding site of PLDH and Heme are responsible for the appropriate anti-plasmodial activity of the CQ analogs. Therefore, these compounds can be considered as new, effective CQ-analogs especially in prevention. The compounds can be promising anti-malarial agents after further studies (e.g. formulation strategies, co-formulation with other antimalarial drugs) and the use of drug delivery systems in order to improve their pharmacokinetics.

Abbreviations

Ala: Alanine ; ACTs: Artemisinin combination therapies; ART: Artemisinin; Asp: Aspartic acid; BALB/c: laboratory-bred strain of the [house mouse](#); CQ: Chloroquine; Gly: Glycine; GMS: Greater Mekong Sub-region; IC50: median inhibitory concentration; Ile: Isoleucine; JPEG: Joint photographer's expert group; 1LDG: Crystal structure of PfLDH; Lys: *Lysine*; mg/kg: milligram/kilogram; PfLDH: *Plasmodium falciparum* lactate dehydrogenase; PDB: Protein data bank: 3P5Q: crystal structures of haemoglobin; SPSS: Statistical Package for Social Sciences; Thr: Threonine; Tyr: Tyrosine; WHO: World Health Organization

Declarations

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Ethics approval and consent to participate

The anti-malarial study was performed based on the approval of the Ethics Committee of Pasteur Institute of Iran in accordance with the "Principles of Laboratory Animal Care" (NIH Publication No.85; rev. 1985).

Consent for publication

All authors have reviewed the manuscript and consented for publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

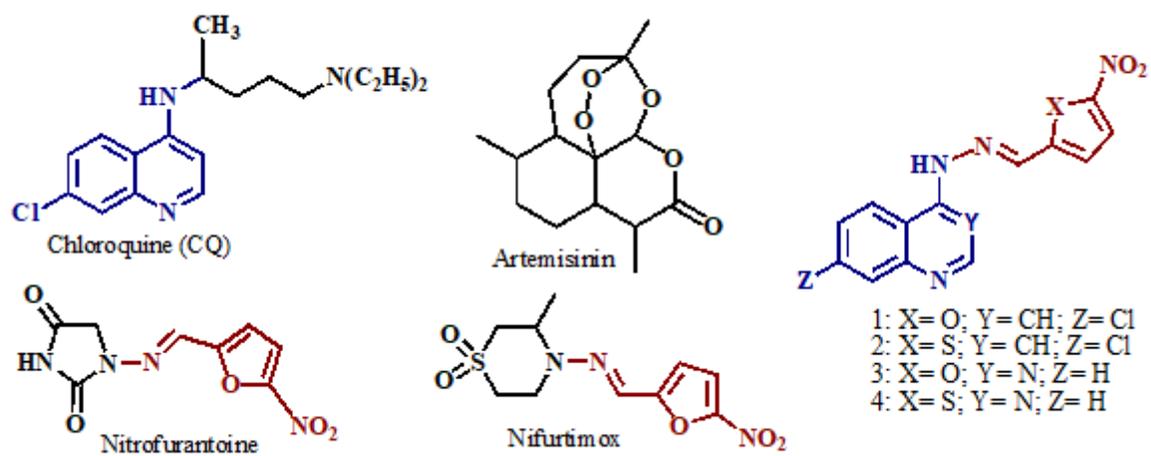


Figure 1

References and experimental compounds

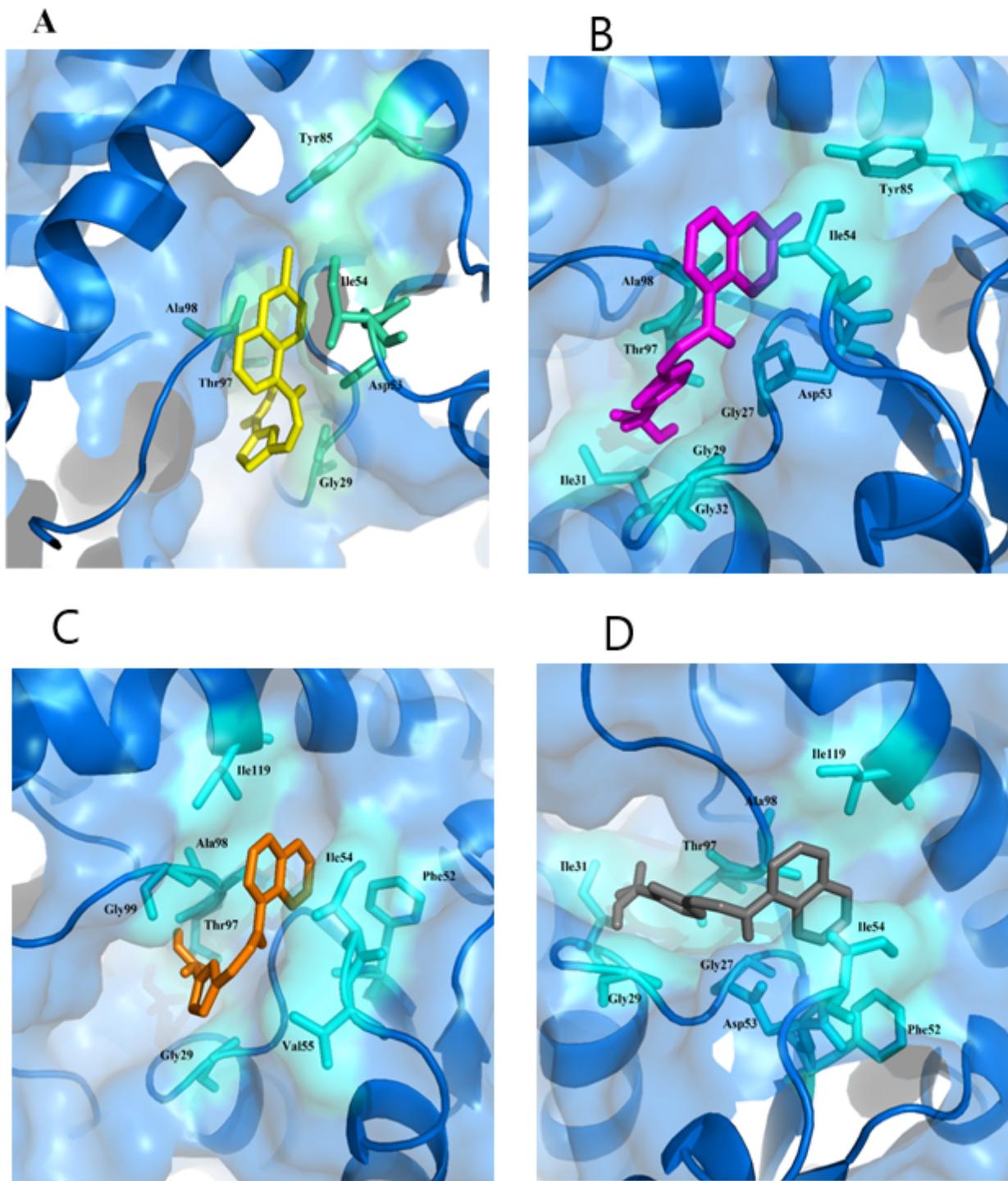


Figure 2

Residues involved in the L-lactate dehydrogenase and ligands interactions. Surface representation of L-lactate dehydrogenase was shown in blue. (A) Interacting residues of L-lactate dehydrogenase with ligand1 (yellow). (B) Interacting residues of L-lactate dehydrogenase with ligand2 (magenta). (C) Interacting residues of L-lactate dehydrogenase with ligand3 (orange). (D) Interacting residues of L-lactate dehydrogenase with ligand4 (gray)

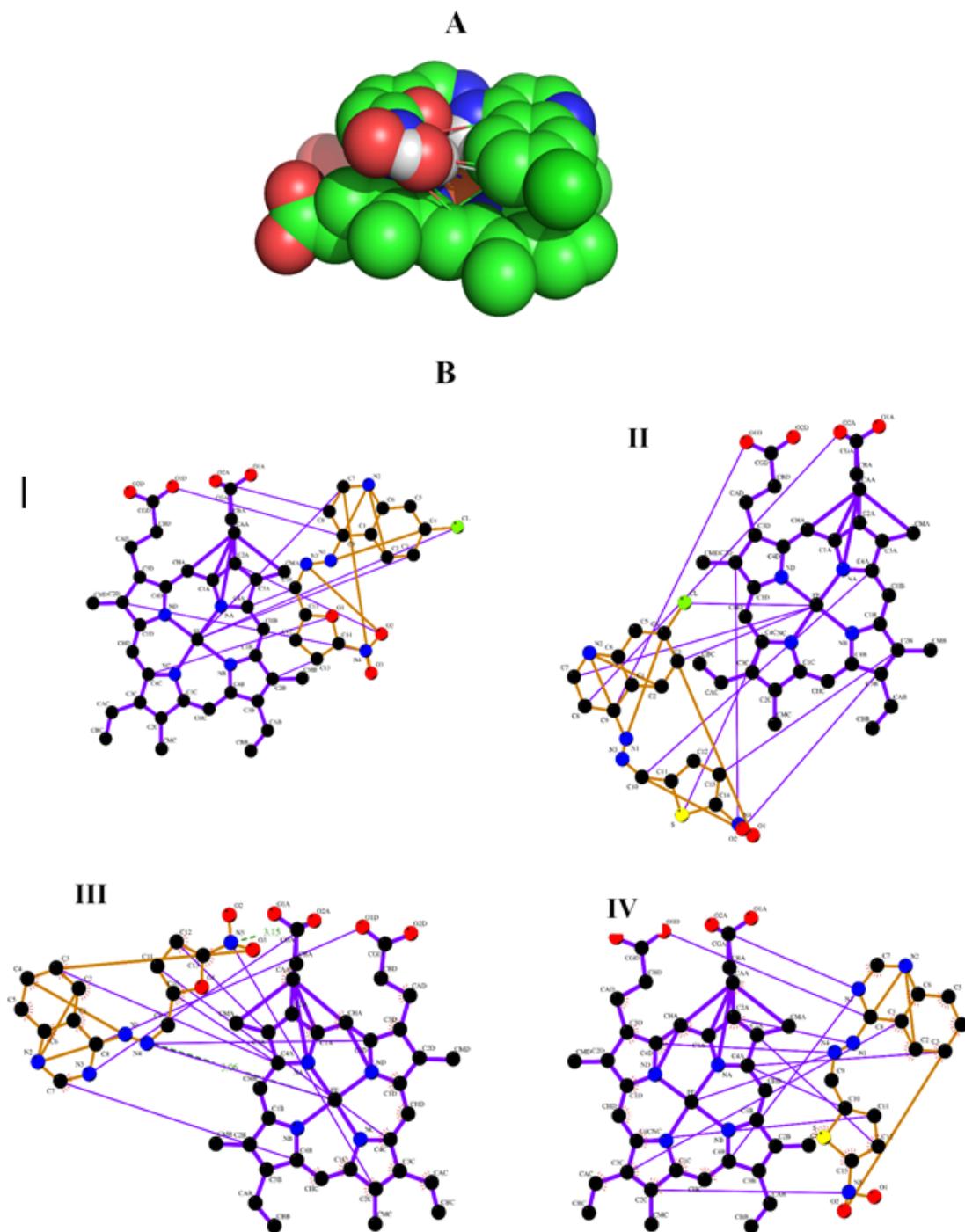


Figure 3

Representation of interaction of heme with ligands. (A) Illustration of heme in complex with ligand. (B) the Ligplot analysis for heme and ligands interaction. Heme-lig1, lig2, lig3 and lig4 complexes were shown in I, II, III and IV, respectively. Heme was shown in magenta and ligands in orange. Green dashed lines were illustrated hydrogen bonds

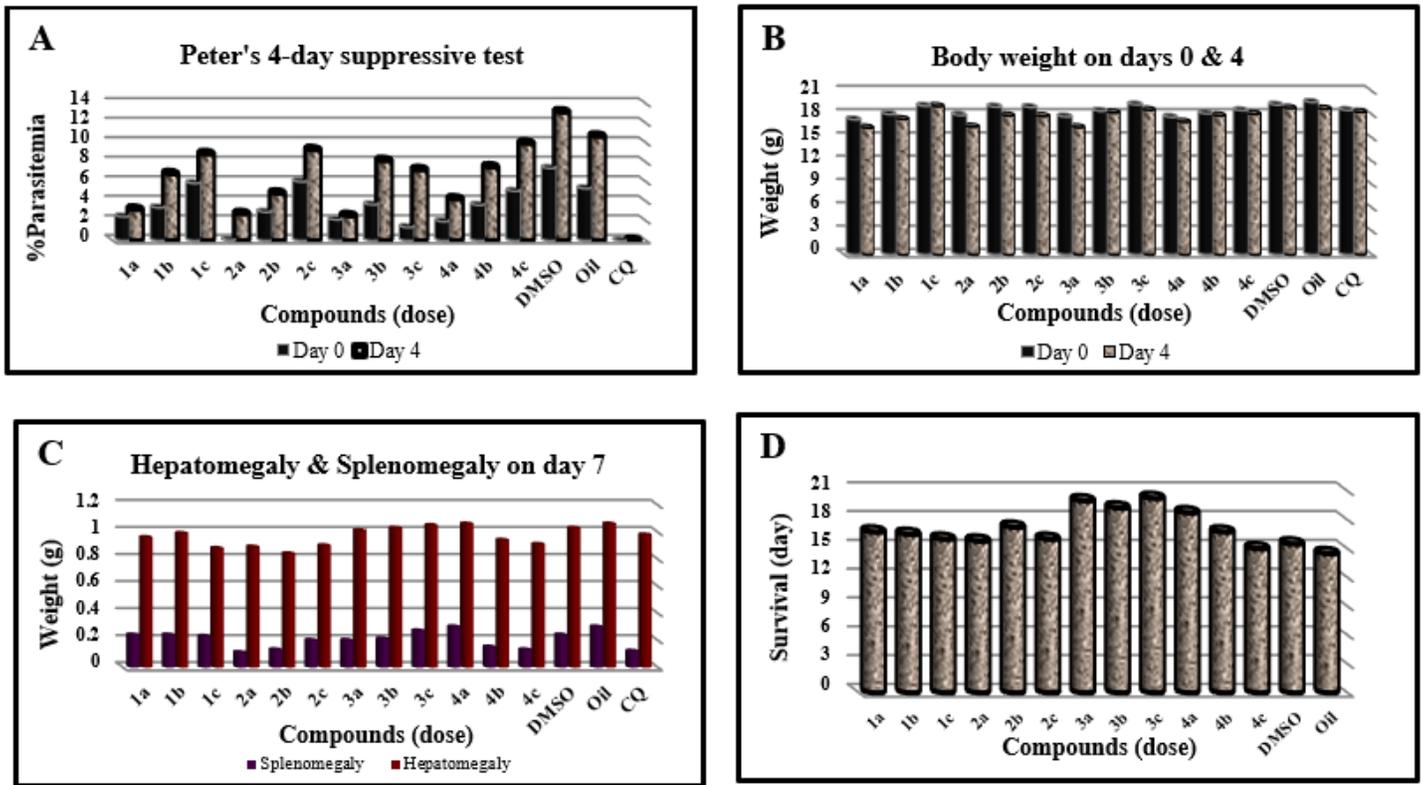


Figure 4

Anti-plasmodial activity of the synthetic compounds in early infection of mice (4-day suppressive test) at various dose (a: 125, b: 62.5, and c: 31.25 mg/kg). (A) %Parasitemia of infected mice on day 0 and 4 (B) Body weight of *P. berghei* infected mice on day 0 and 4 (C) Toxicity of internal organs (D) Mean survival time

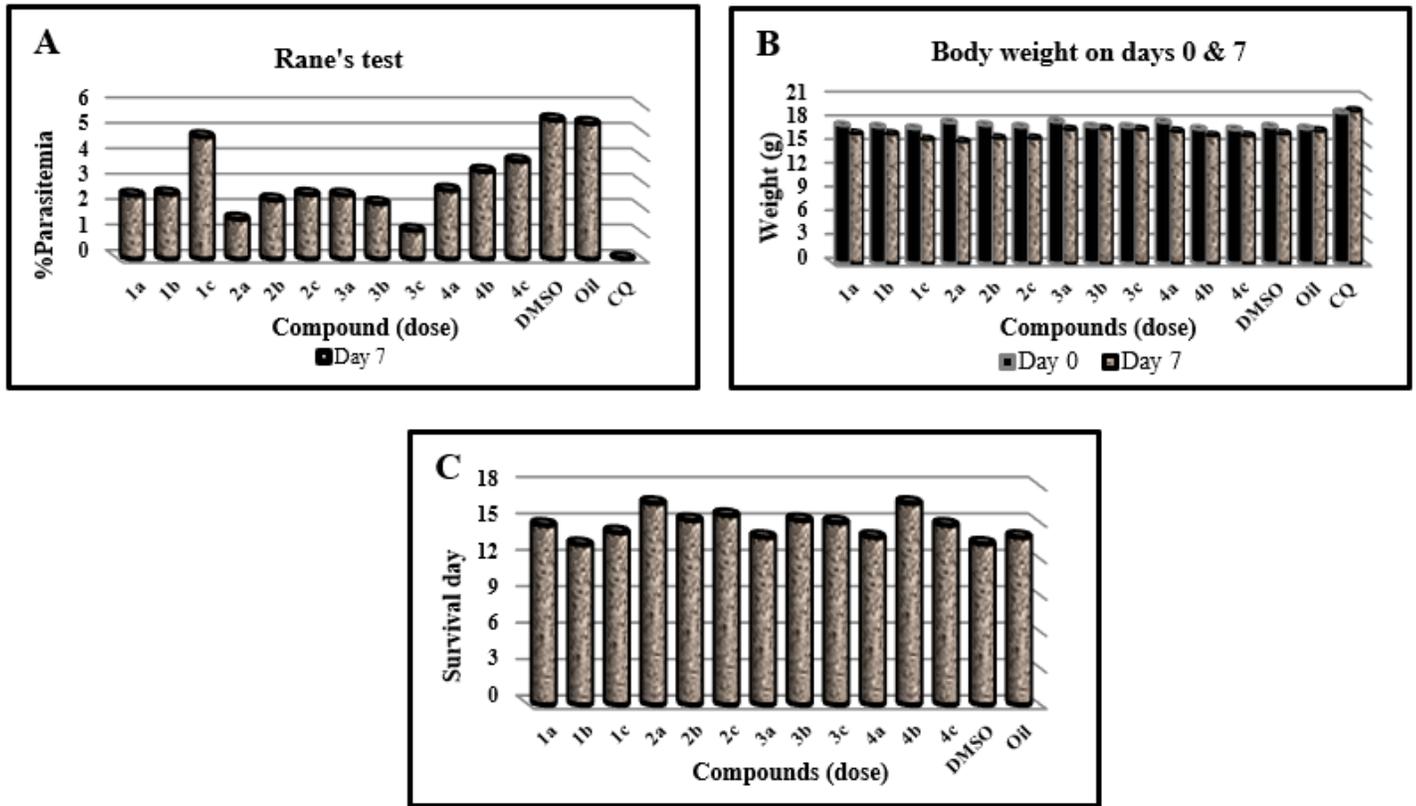


Figure 5

Anti-plasmodial activity of the synthetic compounds in establish infection of mice (Curative Test) at various dose (a:125, b: 62.5, and c: 31.25 mg/kg). (A) %Parasitemia of infected mice on day 7 (B) Body weight of *P. berghei* infected mice on day 0 and 7 (C) Mean survival time

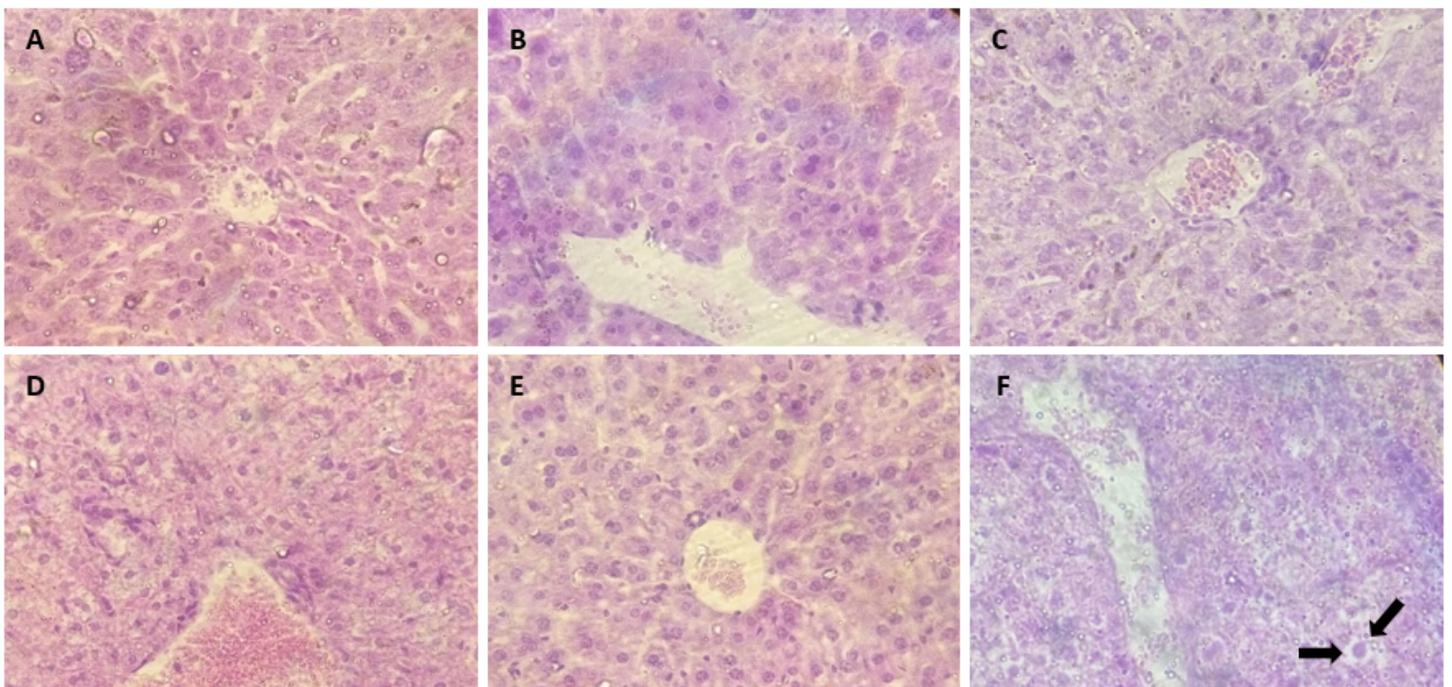


Figure 6

Liver segments of a mouse treated with A: compound 1(125 mg/kg); B: compound 2 (125 mg/kg); C: compound 3 (125 mg/kg); D Compound 4 (125 mg/kg); E: oil, F: CQ (25 mg/kg) on the seventh day. The arrows indicate single cell necrosis.

Supplementary Files

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