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Synthesis and antiplasmodial activity of novel phenanthroline derivatives: An *in vivo* study

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ABSTRACT

Objective(s): Due to the rapid increased drug resistance to Plasmodium parasites, an urgent need to achieve new antiplasmodial drugs is felt. Therefore, in this study, the new synthetic phenanthroline derivatives were synthesized with antiplasmodial activity.

Materials and Methods: A series of 1,10-phenanthroline derivatives containing amino-alcohol and amino-ether substituents were synthesized via facile procedures, starting with 5,6-epoxy-1,10-phenanthroline. Their antiplasmodial activity was then evaluated using Peter's 4-day suppressive test against *Plasmodium berghei*-infected mice (ANKA strain). Furthermore, the mean survival time of the mice treated with synthetic compounds was compared with the negative control group.

Results: The results demonstrated that the compounds 6-(3-(dibutylamino)propylamino)-5,6-dihydro-1,10-phenanthroline-5-ol (**7b**) at the dose of 150 mg/kg/day and 4-(1,10-phenanthroline-5-yloxy)-*N*,*N*-dipropylbutan-1-amine (**8b**) at the dose of 15 mg/kg/day have 90.58% and 88.32% suppression, respectively. All synthetic compounds prolonged the mean survival time of treated mice in comparison with negative control groups, indicating the *in vivo* antiplasmodial activity of these new compounds.

Conclusion: The present study is the first attempt to achieve new, effective synthetic compounds based on phenanthroline scaffold with the antiplasmodial activity. However, more research is needed to optimize their antimalarial activity.

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Introduction

Malaria is one of the most important parasitic diseases worldwide, which is transmitted by female anopheles mosquitoes. Based on WHO reports in 2015, 95 countries had ongoing malaria transmission with an estimated 3.2 billion people at the risk of malaria, especially *Plasmodium falciparum*, as the most deadly malaria parasite in the world (1). Furthermore, there were an estimated 214 million new cases of malaria and 438,000 deaths annually, which are mostly children. Despite many efforts to control, eliminate, and eventually eradicate this infection, malaria still remains the greatest global health problem. However, for malaria control, there are various methods such as personal protection, mosquito control using insect repellents and insecticides, malaria prophylaxis, and treatment with antiplasmodial drugs. In fact, the initial detection and treatment of the disease by itself are sufficient for the control of this epidemic infection, at least at its early stages. By these preventive actions, the parasite load in the community is decreased, thereby reducing the transmission of the disease.

Drug therapy is one of the main methods of malaria control. There are some drugs that affect different stages (exoerythrocytic, erythrocytic, and sexual) of the parasite's life cycle. For instance, chloroquine (CQ), mefloquine (MQ), amodiaquine (AQ), and halofantrine (HAL) are effective drugs in parasite's erythrocytic stage that interfere with detoxification mechanism of the parasite (Figure 1) (2). These drugs belong to the family of quinoline analogs. Actually, CQ and AQ are 4-aminoquinoline derivatives, whereas MQ and HAL are aryl-amino alcohols derivatives. All these drugs have already been used in malaria control, elimination, and eradication programs because of their easy usage, affordable synthesis, or great clinical efficacy. Some of them are also safe for children and pregnant women. Nevertheless, in recent years, the value of these drugs for the prevention and treatment of malaria has decreased after development and the spread of drug resistance, especially against quinoline analogs (3, 4). Indeed, the re-emerging of malaria in many endemic areas of the world is attributed to the rapid increase of resistance to available antiplasmodial drugs and the

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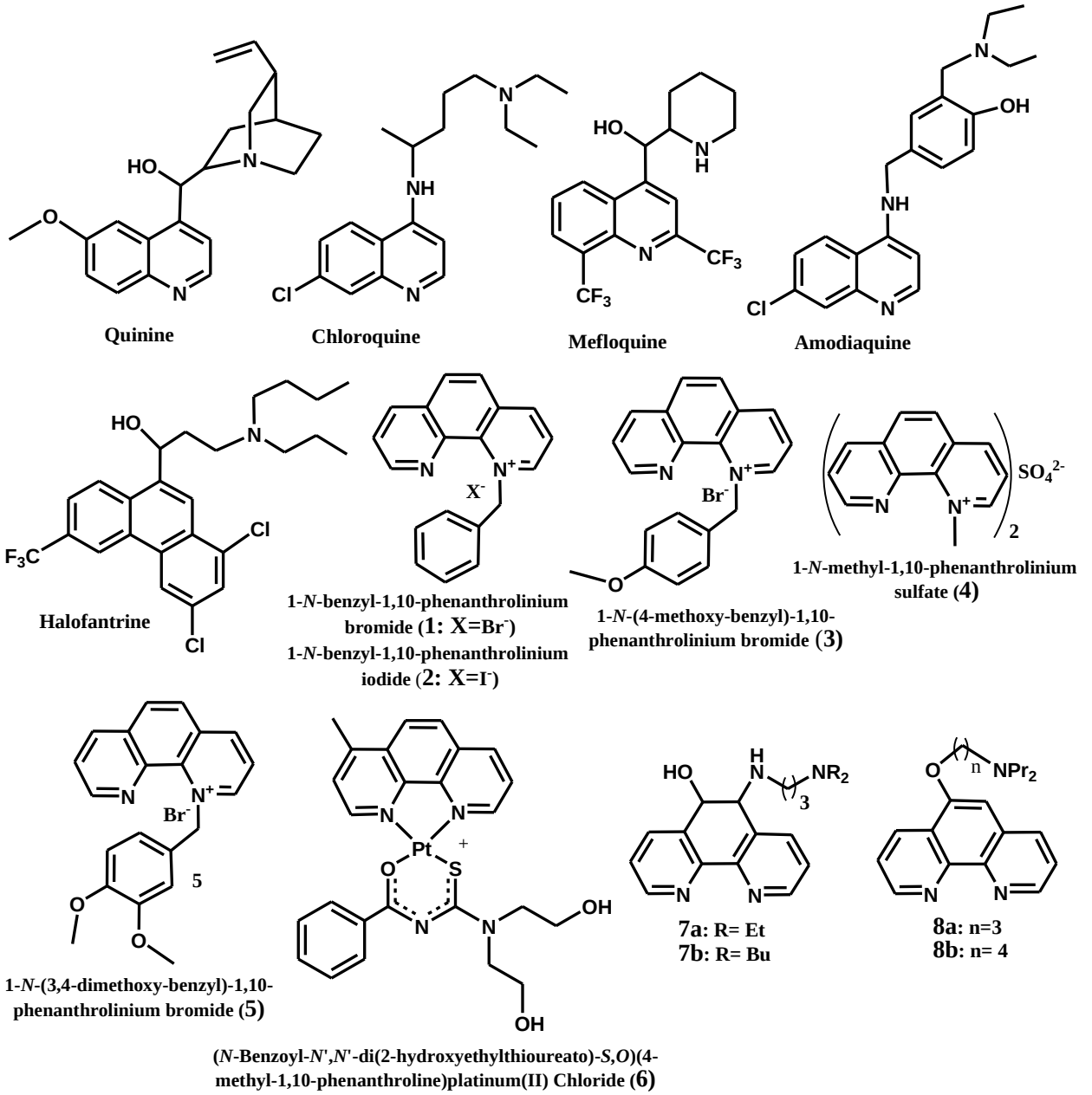


Figure 1. Antimalarial drugs (quinine, chloroquine, mefloquine, amodiaquine, and halofantrine), synthetic compounds with phenanthroline scaffold (1-*N*-benzyl-1,10-phenanthrolinium bromide (**1**); 1-*N*-benzyl-1,10-phenanthrolinium iodide (**2**); 1-*N*-(4-methoxy-benzyl)-1,10-phenanthrolinium bromide (**3**); 1-*N*-methyl-1,10-phenanthrolinium sulfate (**4**); 1-*N*-(3,4-dimethoxy-benzyl)-1,10-phenanthrolinium bromide (**5**); (*N*-Benzoyl-*N*',*N*'-di(2-hydroxyethylthioureato)-*S*,*O*)(4-methyl-1,10-phenanthroline)platinum(II) chloride (**6**), and designed compounds (amino-alcohol and amino-ether phenanthroline derivatives **7a-7b** and **8a-8b**)

resistance of vectors to insecticides. As an example, *P. falciparum* is extremely resistant to CQ and MQ in the areas where these drugs are used widely (5). In addition, AQ resistance has been reported in South America, Asia, and East Africa (6). It is noticeable that there is cross-resistance between these quinoline drugs due to the similarity of their chemical structures (7).

Considering the resistance problem to CQ and its quinoline analogs, a new drug with different scaffolds, known as HAL, was discovered. HAL was primarily purposed for healthy people, to protect them from malaria (8). This aryl-amino alcohol derivative with phenanthrene scaffold is effective against CQ and multi-drug-resistant *P. falciparum* malaria. But, its use is limited to malaria treatment due to the risk of toxicity and unreliable absorption. On the other hand, development of MQ resistance resulted in cross-resistance to HAL, thus reducing its usage (9).

Artemisinins, as the best antimalarial drugs in the current situation, showed very rapid parasite clearance

times. Since artemisinins have a short half-life and are fast acting, artemisinin-based combination therapy (ACT), especially with a different class of long-lasting antimalarial drugs, has been recommended for treating *P. falciparum* malaria (10). Recently, resistance to ACTs has been reported in Asian countries, which can be the start of a catastrophic incidence in the world (11). It is remarkable that drug resistance can lead to malaria prophylaxis and treatment failure in the absence of an alternative, tolerable and safe drug, particularly for children and pregnant women. Therefore, pharmaceutical companies and academic researchers have focused on the development of novel antiplasmodial drugs. In this light, these groups considered two main features for drug discovery: first, discovery of new natural products with antimalarial activity and second, the achievement of new synthetic medicines with activity against the strains of the parasite, which is a powerful tool for malaria control (12).

Drug development based on synthetic methods plays a vital role in modern drug discovery, and in this concern, the identification of lead compound is very important. For instance, chloroquine was designed and synthesized based on quinine, as an identified natural product, for the purpose of decreasing quinine side effects (3). Other quinoline analogs (such as AQ, MQ, and HAL) were also prepared with the replacement of the side chain or aromatic ring to overcome drug resistance and to enhance desired physiochemical or biological properties.

Due to the importance of aromatic or heteroaromatic scaffolds in medicinal chemistry, other new compounds with different scaffolds were synthesized and evaluated in antiplasmodial tests (13). The 1,10-phenanthroline is one of these heteroaromatic scaffolds that is considered as diaza-analog of phenanthrene with two nitrogen atoms at C-1 and C-10 positions and quinoline analog with a fused pyridine ring. Therefore, considering the side effects, high cost, and unreliable absorption of HAL, the researchers synthesized its diaza-analogs by the replacement of phenanthrene with 1,10-phenanthroline and evaluated their antiplasmodial activities in both *in vitro* and *in vivo* tests (Figure 1) (14-20).

In the present study, with regard to the spread of resistance to quinoline antiplasmodial drugs, their disadvantages, and the great potential of 1,10-phenanthroline (14-19), four new phenanthroline derivatives were synthesized and evaluated for the first time against *Plasmodium berghei* (ANKA strain). Similar to the available antiplasmodial drugs, these derivatives were composed of aliphatic side chain containing tertiary amine. They were synthesized from 5,6-epoxy-1,10-phenanthroline as a starting agent. As shown in Figure 1, the phenanthroline derivatives are divided into two groups, amino-alcohol, and amino-ether phenanthroline compounds. The antiplasmodial activity of the synthetic compounds was also assessed by Peter's test in mice inoculated with *P. berghei*. Furthermore, the mean survival time of the mice treated with synthetic compounds was compared with the negative control groups.

Materials and Methods

Chemistry

All chemical reagents and materials were purchased from Sigma-Aldrich Company (USA). Solvents were procured from Sumchun Company (South Korea). The key intermediates 5,6-epoxy-1,10-phenanthroline (**9**) and 5-hydroxy-1,10- phenanthroline (**10**) were prepared based on the methods described in literatures (21, 22). Uncorrected melting points were determined on a Kofler hot stage apparatus. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide dicks). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Unity 500 spectrometer, and chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane, as an internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Elemental analyses were carried out on the CHN rapid elemental analyzer (GmbH, Germany) for C, H, and N, and the results were within 0.4% of the theoretical values. Merck silica gel 60 F254 plates were used for analytical TLC. The logP of compounds were performed using ACD/ChemSketch Freeware version.

Table 1. The *in vivo* activities of four synthetic compounds (**7a-b** and **8a-b**) against *Plasmodium berghei*

P-value	Mean survival rate (day)	% Suppression of parasitemia	Average % parasitemia ± SD	Dose (mg/kg)	logP	Compounds	Groups
< 0.0001	22.00	53.47	4.98 ± 0.43	150	1.73	**7a**	**1**
	16.75	27.08	7.80 ± 1.04	100			
	17.75	18.50	8.72 ± 1.49	50			
	22.25	**90.58**	**1.01 ± 0.94**	150	3.18	**7b***	**2**
< 0.0001	18.50	74.63	2.71 ± 1.14	100			
	17.50	22.25	8.32 ± 1.53	50			
	22.00	52.98	5.03 ± 1.15	30	3.83	**8a**	**3**
0.009	20.75	30.89	7.39 ± 1.49	20			
	19.50	13.22	9.28 ± 1.05	10			
	21.67	**88.32**	**1.25 ± 1.24**	15	4.28	**8b***	**4**
< 0.0001	21.00	47.94	5.57 ± 1.13	12.5			
	18.75	17.17	8.86 ± 1.34	10			
	15.50	0	10.47± 2.42	-	-	**PBS**	**5**
	16.00	0	10.93 ± 2.075	20 %	-	**DMSO**	**6**
	-	100	-	25	-	**CQ**	**7**

SD: Standard Deviation; * shows the most potent compounds

Synthesis of 6-(3-(diethylamino)propylamino)-5,6-dihydro-1,10-phenanthroline-5-ol (7a)

A mixture of 0.2 g (1.02 mmol) 5,6-epoxy phenanthroline (**9**) and 1.6 ml (10.15 mmol) 3-(diethylamino)propylamine in absolute ethanol was refluxed at 80 °C for 24 hr. The completion of the reaction was detected by TLC, and the solvent was removed under reduced pressure to obtain a brown solid. The solid was dissolved in dichloromethane (100 ml) and washed with aqueous NaOH 10%. Then the organic layer was separated and washed with brine (30 ml) and was dried using sodium sulfate. The filtrated organic layer was concentrated by a rotary evaporator. The final product was purified by silica gel column chromatography (dichloromethane/ethanol) for obtaining a cream solid.

Synthesis of 6-(3-(dibutylamino)propylamino)-5,6-dihydro-1,10-phenanthroline-5-ol (7b)

A mixture of 0.2 g (1.02 mmol) 5,6-epoxy phenanthroline (**9**) and 2.4 ml (10.65 mmol) 3-(dibutylamino) propylamine in absolute ethanol was stirred at room temperature for eight days. The completion of the reaction was detected by TLC and the solvent was removed under reduced pressure to obtain a brown grassy solid. The solid was dissolved in dichloromethane (100×2 ml) and washed with aqueous NaOH 10%. The organic layer was then separated and washed with brine (30 ml) and dried using sodium sulfate. The filtrated organic layer was concentrated by a rotary evaporator. The final product was purified by silica gel column chromatography (dichloromethane/ethanol) to obtain a cream solid.

General procedure for the synthesis of intermediates 11a-b

A mixture of 0.11 g (4.58 mmol) sodium hydride and 0.2 g (1.02 mmol) 5-hydroxy-1,10-phenanthroline (**10**) in 15 ml of ethanol was stirred vigorously at room temperature for 30 min. The mixture was then added to the solution of dibromo alkyl (5.96 mmol) in dry THF dropwise; this mixture was refluxed for ~3-4 hr. The completion of the reaction was detected by TLC. After filtration, the solvents were removed under reduced pressure to obtain a yellow viscose solid. Excess of dibromo alkyl was removed by hot petroleum ether, and finally, a cream solid was obtained.

General procedure for the synthesis of compounds 8a-b

A volume of 0.65 mmol bromoalkoxy-1,10-phenanthroline (**11a-b**), 2.5 mmol dipropylamine, and 3 g potassium carbonate were mixed in 20 ml of absolute ethanol, and the mixture was then refluxed for ~24-28 hr. The completion of the reaction was detected by TLC, and the solvent was removed under reduced pressure to obtain a dark yellow viscose solid. The residue was decanted with H_2O and chloroform. The organic phase was separated, and the solvent was removed under reduced pressure to obtain a yellow solid. The solid was purified by silica gel column chromatography (ethyl acetate/petroleum ether) until a cream solid was obtained.

Evaluation of antiplasmodial activity (Peter's test)

The experimental female BALB/c mice (6-8 weeks) were purchased from Pasture Institute of Iran (Tehran) and were kept under standard conditions for ten days to adapt to the laboratory animal housing facilities. The synthetic compounds, **7a-b** and **8a-b**, were administered intraperitoneally to three female BALB/c mice for 5 days with the concentrations of 10 to 150 mg/kg/day. The signs of mortality in each group were monitored daily. The optimum dose of compounds **7a-b** was 150 mg/kg/day, while for compounds **8a** and **8b** were 30 and 15 mg/kg/day, respectively. The antiplasmodial (schizontocidal) activity of synthetic compounds (**7a-b** and **8a-b**) was evaluated using the 4-day suppressive test against *P. berghei* infection in mice (23). The 19-22 g mice were weighed and randomized into seven groups and again weighted after the experiment. The stock of CQ-sensitive *P. berghei* (ANKA) parasite (500 µl containing 25% *P. berghei*) was defrosted and injected into two female BALB/c mice. Next, five animals were selected and infected with *P. berghei* through passaging. Each animal was inoculated IP with 2×10^7-infected erythrocytes of *P. berghei* in PBS (200 µl) on the first day (D0) of the experiment. The compounds were solubilized in 20% DMSO and prediluted in PBS to make appropriate concentrations. The first treatment was accomplished three hours after the mice were infected (D0) and treated daily for four consecutive days (D4). Groups 1 and 2 were treated with compounds **7a** and **7b** (50, 100, and 150 mg/kg/day) by IP injection for four days, whereas groups 3 and 4 were treated with compounds **8a** (10, 20, and 30 mg/kg/day) and **8b** (10, 12.5, and 15 mg/kg/day) (Table 1). Mice groups 5 and 6 received PBS and 20% DMSO as negative controls, and mice group 7 was treated by CQ (25 mg/kg/day), as a positive control, for four days (Table 1). On day four, tail blood smears were taken, stained with 10% Giemsa stain in phosphate buffer (pH 7.2) for 20 min and then visualized under a microscope at 100× magnifications to determine the parasitemia level. The parasitized red blood cells on at least 2,000 red blood cells were counted to calculate the percentage of parasitemia (%parasitemia = the number of infected RBC/the total number of RBC ×100). The percentage of parasitemia suppression for each group was evaluated by comparing the percentage of parasitemia in negative controls with that in the treated group (%suppression = parasitemia in negative control - parasitemia in treated group/parasitemia in negative control ×100). During the treatment, all mice were weighed on days 0 and 4. Also, the dissection of the internal organs (spleen, liver, and kidney) was done on

the seventh day of treatment. The kidneys of the treated groups did not show any change. Furthermore, the mortality of mice was monitored daily during experiment up to 24 days post-infection, and the mean survival rate of each group was calculated.

Statistical analysis

Control and test data were analyzed using SPSS (version 22.0, 2012). One-way ANOVA was used to test the statistical differences for three doses within a group, followed by LSD and Tukey's test for pairwise comparisons. $P \leq 0.05$ was considered statistically as significant.

Results

Chemistry

The pathway for the synthesis of compounds **7a-b** is shown in Scheme 1. The intermediate of 5,6-epoxy-1,10-phenanthroline (**9**) was obtained from the reaction of 1,10-phenanthroline with aqueous sodium hypochlorite (21, 24; Scheme 1). The reaction of 5,6-epoxy-1,10-phenanthroline (**9**) with alkyl diamines in absolute ethanol gave compounds **7a-b** in good yields. Indeed, epoxide is reactive due to the ring strain and can easily react with alkyl diamines through nucleophilic attack. It is remarkable that the epoxide ring opening is stereospecific, and nucleophilic attack with inversion gives *trans* product. The compound **7a**, the hydrogens of the phenanthroline nucleus, indicated a triplet at 8.62 ppm (H_1 and H_8 in phenanthroline), doublet at 7.90 ppm (H_3 in phenanthroline), and multiplet at 7.40 ppm (H_2 and H_7 in phenanthroline). The compound **7a** had a *trans* format, which was confirmed by a doublet at 4.74 ppm (H_4 in phenanthroline) and a doublet at 3.80 ppm (H_5 in phenanthroline) with coupling constant ~9.5 Hz.

The spectral data confirmed the structure of the derivatives. In ^{1}HNMR spectra of compound **7b**, the hydrogens of the phenanthroline nucleus showed a broad singlet at 8.72 ppm (H_1 and H_8 in phenanthroline), two sets of doublet at 8.03 ppm (H_3 in phenanthroline) and 7.88 ppm (H_6 in phenanthroline), and a multiplet at 7.31 ppm (H_2 and H_7 in phenanthroline). Also, a doublet at~4.84 ppm (H_4 in phenanthroline) and a doublet at~3.92 ppm (H_5 in phenanthroline) were detected with coupling constant~10.5 Hz, which confirms the formation of *trans* product. In the ^{1}HNMR spectra of compounds **7a-b**, the aliphatic hydrogens in the side chain on the phenanthroline nucleus were recognizable regarding their spin-spin splitting patterns.

The synthesis of target compounds **8a** and **8b** is outlined in Scheme 1. As shown in the Scheme, the intermediate of 5-hydroxy-1,10-phenanthroline (**10**) was obtained from 5,6-epoxy-1,10-phenanthroline based on the method reported previously (22). The intermediates of bromoalkoxy-1,10-phenanthroline (**11a-b**) were obtained from the reaction of compound **10** in the presence of sodium hydride, as a strong and a solid base in dry ethanol, which can deprotonate the hydroxyl group. This mixture was then added to excess dibromo alkyl in dry tetrahydrofuran and refluxed. The reaction of intermediates **11a-b** in absolute ethanol with dipropylamine in the presence of excess potassium carbonate afforded the final compounds (**8a-b**).

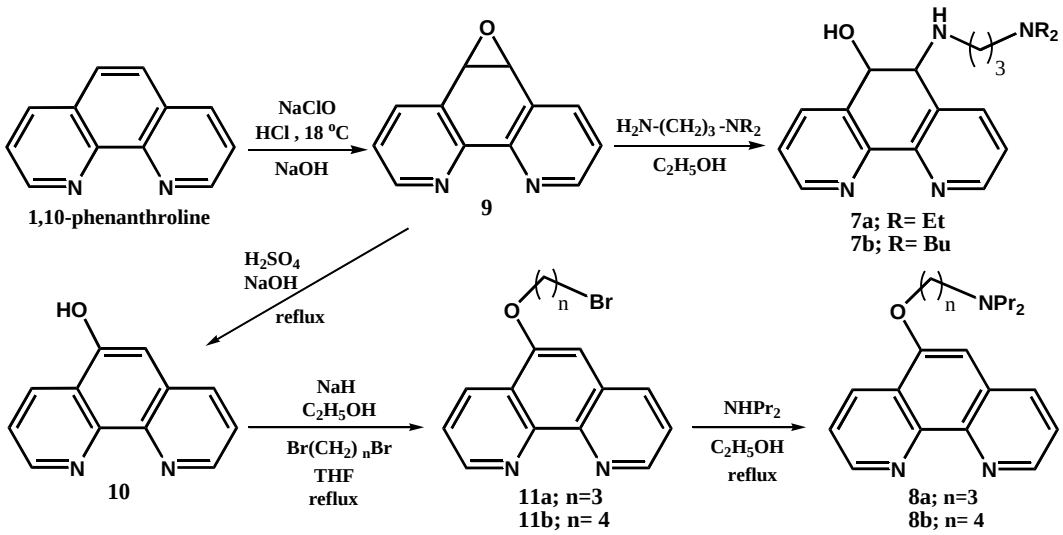
The ^{1}HNMR spectra of compound **8a**, the hydrogens of the phenanthroline nucleus in DMSO-d6 showed a doublet at 9.12 ppm (H_1 in phenanthroline), a broad singlet at 8.91 ppm (H_7 in phenanthroline), a doublet-doublet at 8.67 ppm (H_3 in phenanthroline), a triplet at 8.33 ppm (H_5 in phenanthroline), two sets of multiplet at 7.79 and 7.67 ppm (H_2 and H_6 in phenanthroline), and a singlet at 7.37 ppm (H_4 in phenanthroline). The compound **8b**, the hydrogens of the phenanthroline nucleus, indicated two sets of triplet at 9.19 and 9.00 ppm (H_1 and H_7 in phenanthroline), a doublet-doublet at 8.68 ppm (H_3 in phenanthroline), a doublet at 8.07 ppm (H_5 in phenanthroline), two sets of multiplet at 7.63 and 7.54 ppm (H_2 and H_6 in phenanthroline), and a singlet at 6.92 ppm (H_4 in phenanthroline) in its ^{1}HNMR spectra in $CDCl_3$. The aliphatic hydrogens in the side chain on the phenanthroline nucleus of compounds **8a-b** were recognizable with regards to their spin-spin splitting patterns in ^{1}HNMR spectra. Finally, the formation of all synthetic compounds was confirmed by different analysis methods, including ^{13}CNMR, Mass, and CHN analysis.

6-(3-(diethylamino)propylamino)-5,6-dihydro-1,10-phenanthroline-5-ol (7a)

Yield: 78%, m.p. > 300 °C. ^{1}HNMR($CDCl_3$, 500 MHz) δ: 8.62 (t, 2H, *J*= 8 Hz, phen), 7.90 (d, 2H, *J*= 8 Hz, phen), 7.40 (m, 2H, phen), 5.02 (br s, 1H, -OH), 4.74 (d, 1H, *J*= 9.5 Hz, phen), 3.80 (d, 1H, *J*= 9.5 Hz, phen), 2.80 (br s, 1H, -NH), 2.60 (t, 2H, *J*= 7 Hz, -CH_2-), 2.38 (m, 4H, *J*= 7 Hz, -CH_2-), 1.52 (t, 2H, *J*= 7 Hz, -CH_2-), 0.93 (t, 6H, *J*=7 Hz, -CH_3). Anal.Calcd for $C_{19}H_{26}N_4O$: C, 69.91; H, 8.03; N, 17.16. Found: C, 69.73; H, 7.71; N, 17.09.

6-(3-(dibutylamino)propylamino)-5,6-dihydro-1,10-phenanthroline-5-ol (7b)

Yield: 58%, m.p. > 300 °C. ^{1}HNMR($CDCl_3$, 500 MHz) δ: 8.72 (br s, 2H, phen), 8.03 (d,1H, *J*= 8 Hz, phen), 7.88 (d,1H, *J*= 8 Hz, phen), 7.31 (m, 2H, phen), 4.84 (d, 1H, *J*= 10.5 Hz, phen), 3.92 (d, 1H, *J*= 10.5 Hz, phen), 2.95 (t, 2H, *J*= 6 Hz, -CH_2-), 2.56 (t, 2H, *J*= 6.5 Hz, -CH_2-), 2.42 (m, 4H, *J*= 7 Hz, -CH_2-), 1.74 (m, 2H, *J*= 6.5 Hz, -CH_2-), 1.42 (m, 4H, *J*= 7 Hz, -CH_2-), 1.30 (m, 4H, *J*= 7.5 Hz, -CH_2-), 0.91 (t, 6H, *J*= 7.5 Hz, -CH_3). MS (m/z, %) = 383.4 [M^+, 31], 339.4 (2), 240.2 (6), 210.2 (6), 181.2 (53), 142.2 (100), 100.2 (73), 70.1 (6), 41.1 (33). Anal.Calcd for $C_{23}H_{34}N_4O$: C, 72.21; H, 8.96; N, 14.65. Found: C, 72.37; H, 8.76; N, 14.86.



Scheme 1. Synthetic route for the preparation of compounds **7a-b** and **8a-b**

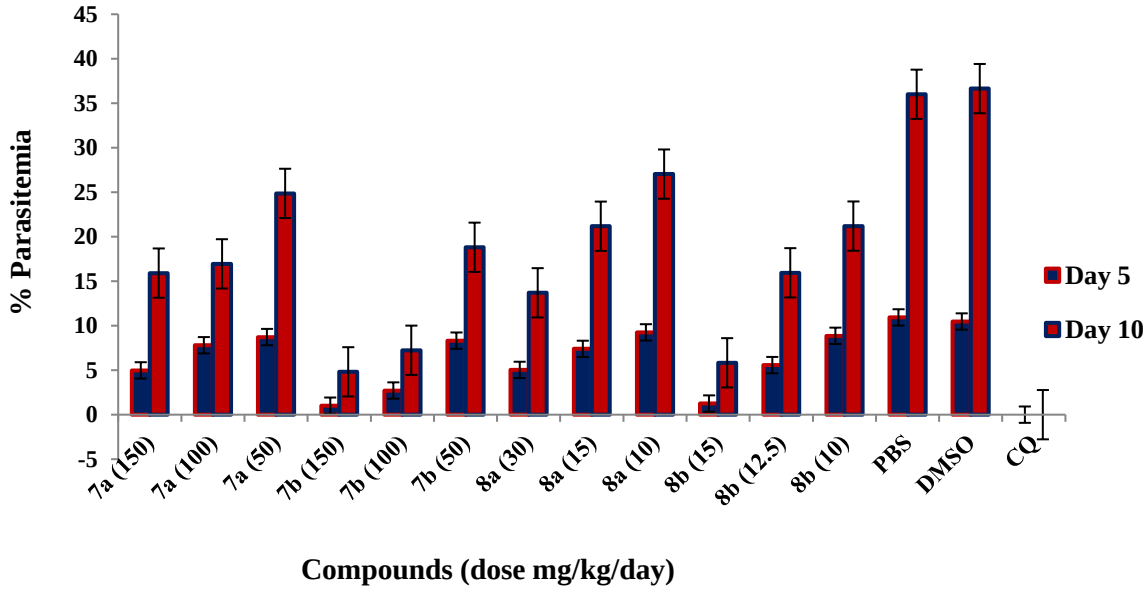


Figure 2. The effect of synthetic compounds (**7a-b** and **8a-b**) intraperitoneally in different doses on the percentage of parasitemia of *Plasmodium berghei*-infected mice (ANKA strain) on days 5 and 10 using the Peter's 4-day suppressive test

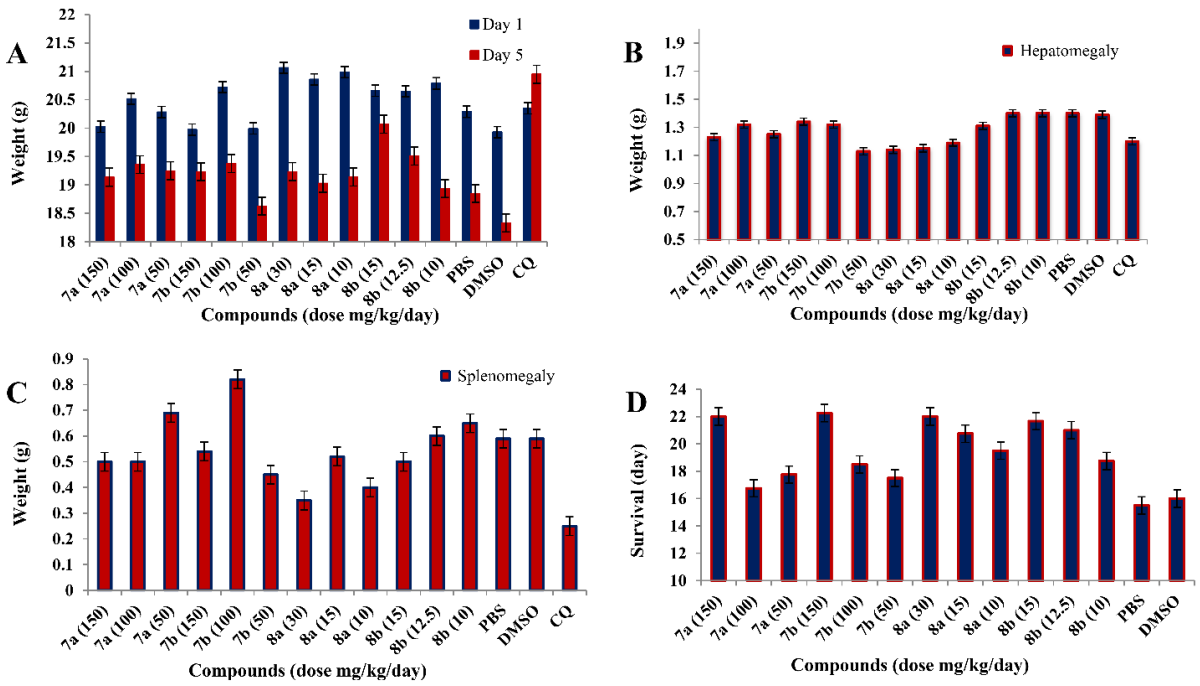


Figure 3. Toxicity assay of treated mice with different doses of drugs (**7a-b** and **8a-b**), including (A) body weight on days 1 and 5, (B) hepatomegaly on day 7, (C) splenomegaly on day 7, and (D) the survival rate up to 24 days post infection

5-(3-bromopropoxy)-1,10-phenanthroline **(11a)**
Yield: 85%, m.p. > 300 °C. ^{1}HNMR(DMSO-d6, 500 MHz) δ: 9.15 (d, 1H, *J*= 4.5 Hz, phen), 8.98 (d, 1H, *J*= 4.5 Hz, phen), 8.74 (dd, 1H, *J*= 6.5 & *J*=1.5 Hz, phen), 8.38 (dd, 1H, *J*= 6.5 and *J* =1.5 Hz, phen), 7.81 (m, 1H, *J*= 4.5 Hz, phen), 7.71 (m, 1H, *J*= 4.5 Hz, phen), 7.40 (s, 1H, phen), 4.42 (t, 2H, *J*= 6.5 Hz, -CH_2-), 3.84 (t, 2H, *J*= 6.5 Hz, -CH_2-), 2.49 (m, 2H, *J*= 6.5 Hz, -CH_2-).^{13}CNMR(DMSO-d6, 500 MHz) δ: 153.54, 153.50, 150.36, 147.49, 147.40, 147.36, 135.01, 130.61, 129.12, 123.44, 123.05, 102.33, 66.19, 31.64, 31.30.

5-(4-bromobutoxy)-1,10-phenanthroline **(11b)**
Yield: 93%, m.p. > 300 °C. ^{1}HNMR($CDCl_3$, 500 MHz) δ: 9.15 (d, 1H, *J*= 4 Hz, phen), 8.96 (d, 1H, *J*= 4 Hz, phen), 8.60 (dd, 1H, *J*= 6.5 and *J*= 1.5 Hz, phen), 8.02 (dd, 1H, *J*= 6.5 and *J*=1.5 Hz, phen),7.60 (q, 1H, *J*= 6.5 Hz, phen), 7.49 (q, 1H, *J*= 6.5 Hz, phen), 6.85 (s, 1H, phen), 4.21 (br s, 2H, -CH_2-), 3.49 (br s, 2H, -CH_2-), 2.05 (br s, 2H, -CH_2-), 1.22 (br s, 2H, -CH_2-).

3-(1,10-phenanthroline-5-yloxy)-N,N-dipropylpropan-1-amine **(8a)**
Yield: 65%, m.p. > 300 °C. ^{1}HNMR (DMSO-d6, 500 MHz) δ: 9.12 (d, 1H, *J*= 3.5 Hz, phen), 8.91 (brs, 1H, phen), 8.67 (dd, 1H, *J*= 8 and *J*= 2.5 Hz, phen), 8.33 (t, 1H, *J*= 3.5 Hz, phen), 7.79 (m, 1H, *J*= 4 Hz, phen), 7.67 (m, 1H, *J*= 4 Hz, phen), 7.37 (s, 1H, phen), 4.41 (t, 2H, *J*= 6 Hz, -CH_2-), 2.89 (t, 4H, *J*= 7.5 Hz, -CH_2-), 2.08 (br s, 2H), 1.55 (t, 4H, *J*= 7.5 Hz, -CH_2-), 0.89 (t, 6H, *J*= 7.5 Hz, -CH_3). ^{13}CNMR (DMSO-d6, 500 MHz) δ: 153.55, 153.50, 150.26, 147.49, 147.40, 147.36, 135.01, 130.62, 129.11, 123.34, 123.01, 102.31, 66.82, 66.15, 46.43, 31.66, 27.42, 19.15, 11.61. MS (m/z, %) = 337.4 [M^+, 3], 308.3 (6), 268.1(54), 236.2 (44), 196.2 (43), 167.2 (72), 140.1 (22), 114.1 (14), 73.2 (29), 45.1 (100). Anal.Calcd for $C_{21}H_{27}N_3O$: C, 74.74; H, 8.06; N, 12.45. Found: C, 74.36; H, 8.31; N, 11.87.

4-(1,10-phenanthroline-5-yloxy)-N,N-dipropylbutan-1-amine **(8b)**
Yield: 54%, m.p. > 300 °C. ^{1}HNMR($CDCl_3$, 500 MHz) δ: 9.19 (t, 1H, *J*= 2.5 Hz, phen), 9.00 (t, 1H, *J*= 2.5 Hz, phen), 8.68 (dd, 1H, *J*= 8 and *J*= 2.5 Hz, phen), 8.07 (d, 1H, *J*= 8 Hz, phen), 7.63 (m, 1H, *J*= 2.5 Hz, phen), 7.54 (m, 1H, *J*= 2.5 Hz, phen), 6.92 (s, 1H, phen), 4.27 (t, 2H, *J*= 6.5 Hz, -CH_2-), 2.62 (t, 2H, *J*= 7 Hz, -CH_2-), 2.53 (t, 4H, *J*= 7 Hz, -CH_2-), 2.07 (m, 2H, *J*= 6.5 Hz, -CH_2-), 1.90 (m, 2H, *J*= 7 Hz, -CH_2-), 1.47 (m, 4H, *J*= 7 Hz, -CH_2-), 0.89 (t, 6H, *J*= 6.5 Hz, -CH_3). MS (m/z, %) = 351.3 [M^+, 25], 322.2 (55), 282.1 (40), 250.1 (37.5), 221.1 (6.25), 196.1 (92.5), 167.1 (65), 140.1 (45), 114.2 (52.5), 87.2 (100), 45.2 (75). Anal.Calcd for $C_{22}H_{29}N_3O$: C, 75.18; H, 8.32; N, 11.96. Found: C, 74.89; H, 8.01; N, 12.15.

In vivo antiplasmodial activity
The compound **8b**, as the amino-ether derivative of 1,10-phenanthroline, showed 88.32% *in vivo* suppression of parasitemia at the low dosage of 15 mg/kg/day by IP route using Peter's 4-day suppressive test against infected *P. berghei* (Table 1 and Figure 2) (23). However, 90.58% suppression was observed for the compound **7b**, as the amino-

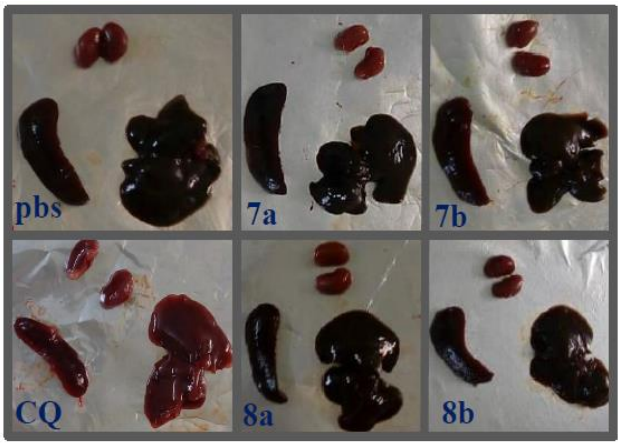


Figure 4. Dissection of the internal organs of mice (spleen, liver, and kidney) after treatment with compounds **7a-b** and **8a-b** on day 7. Kidneys of the treated groups did not show any change

alcohol derivative of 1,10-phenanthroline, at the high dose of 150 mg/kg/day. During the treatment, all mice were weighed on days 0 and 4 (Figure 3A). All treated mice had weight reduction, which can be related to the lack of 100% reduction of parasitemia after treatment with synthetic compounds. Seven days after treatment, one of the mice in each group was randomly selected and dissected. The dissection of the internal organs (spleen, liver, and kidney) presented a mild enlargement of the spleen and liver in the treated groups with compounds **7a-7b** and **8a-8b** (Figures 3B and 3C, and 4) compared with the control groups. The kidneys of the treated groups did not show any change. The mortality of mice after IP administration of the synthetic compounds was also investigated, and all of the treated mice had a survival rate higher than the negative control groups (Table 1 and Figure 3D).

The result of statistical analysis between the groups demonstrated that the compound **7a** in the high dose (150 mg/kg/day) had a significant difference in comparison to other doses (P<0.05) but did not show any difference between the doses of 100 and 50 mg/kg (P>0.05). The compound also indicated that difference between the treated groups and the control groups was statistically significant (P<0.05). The compound **7b** showed a significant difference not only in its three doses but also in the control groups (P<0.05). On the other hand, no difference was found for the compound **8a** between doses of 10 and 20 mg/kg as well as between doses of 20 and 30 mg/kg (P>0.05) among its groups. However, there was a difference between the low concentration (10 mg/kg) and the control groups (P<0.05). The comparison among the three study groups of compound **8b** as well as between these groups and the control group indicated no significant difference (P<0.05).

Discussion

Previous studies have shown that phenanthroline derivatives have antiplasmodial activity (14-20). For instance, the derivatives of *N*-benzyl-1,10-phenanthroline (**1** and **2**) have been demonstrated to have good activity against FCR-3 strain with the IC_{50} values of 0.1 and 0.18 µM, respectively after 72-hr incubation (Figure 1) (16). Indeed, the 1,10-phenanthroline ring has metalloprotease inhibitory activity by chelating metal ions. However, Sholikhah *et al.* (16) have obtained contradictory result when synthesized the compounds with *N*-aryl and *N*-alkyl substitution on 1,10-phenanthroline for blockage of the chelating site. Their results confirmed that the antiplasmodial activity of these compounds did not relate to the chelating capacity. The compounds **1** and **2** are nonpolar because of benzyl substituent and can easily penetrate through the cell membrane. Sholikhah *et al.* study has shown that the activity of *N*-benzyl-1,10-phenanthroline derivatives was higher than that of *N*-alkyl-1,10-phenanthroline derivatives. These compounds have also been evaluated by the classical 4-day suppressive test against *P. berghei* (18). The most potent compound was (1)-*N*-benzyl-1,10-phenanthrolinium iodide (**2**) (LD_{50}= 121.42 mg/kg and ED_{50}= 2.08 mg/kg). Investigations have again revealed that the benzyl group is the most important moiety for antiplasmodial activity. The compound with soft anion conjugate (I^-) has more effective interaction with the cell membrane of the parasite, hence giving a better antiplasmodial activity.

Modification of drug structure is a usual procedure to achieve superior activity and less toxicity. Therefore, the researchers designed and synthesized other 1,10-phenanthrolinium derivatives. The antiplasmodial activity of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide (**3**) against two strains of *P. falciparum*, FCR-3, and D10, have been indicated to have the IC_{50} values of 0.82 and 1.21 µM, respectively (15). The suppression of parasitemia was never complete (100% inhibition of parasite growth), and it had lower activity compared to compounds **1** and **2**. A previous study has presented antiplasmodial activity of (1)-*N*-methyl-1,10-phenanthrolinium sulfate (**4**) with the IC_{50} value of 260 nM and also showed that chloroquine diphosphate was more potent than *N*-alkyl and *N*-benzyl-1,10 phenanthroline derivatives (17). Furthermore, the modified fixed-ratio isobologram method has displayed an *in vitro* additive interaction between the compound **4** and CQ. The compound (1)-*N*-(3,4-dimethoxybenzyl)-1, 10-phenanthrolinium bromide (**5**) was synthesized, and the result of heme polymerization inhibitory activity assay revealed that the IC_{50} value of 3.63 mM had more antiplasmodial activity than CQ (19). The compound **5** has two nitrogens; the positively charged nitrogen interacts with the electronegative oxygen at ferriprotoporphyrin IX, and the other nitrogen (base) reacts with the carboxylic acid group at ferriprotoporphyrin IX.

Thus, the heme polymerization process can be prevented. A complex of 1,10-phenanthroline platinum (II) benzoyl thiourea (**6**) presented a suitable activity against K1 and D10 strains of *P. falciparum* with the IC_{50} values of 488 and 282 nM, respectively (20). The complex showed a strong *in vitro* interaction with ferriprotoporphyrin IX and inhibited β-hematin formation. The strong interaction of the phenanthroline complex with ferriprotoporphyrin IX is attributed to the extended planar structure of phenanthroline ring with delocalized electrons in all of these complexes.

In vitro studies may lead to outcomes that do not relate to the situation occurring around a living organism. Therefore, *in vivo* studies is often apply more than *in vitro* because it is suitable for observing the overall effects of an experiment on a living microorganism.

In vivo evaluation of antimalarial compounds typically begins with the use of rodent malaria parasites, especially in drug discovery. In an extensively studied model of murine malaria, mice are infected with *P. berghei*, which is considered as a strong tool for biological studies in the field of malaria. In fact, *P. berghei* is genetically similar to *P. falciparum* and morphologically to *P. vivax*; therefore, it could be a good template for the study of malaria interventions.

In the present work, new amino-alcohol and amino-ether phenanthroline derivatives were synthesized and represented satisfactory results in inhibiting the parasitemia of *P. berghei* infection in BALB/c mice, though the reduction of parasitemia was never completed. Table 1 illustrates the mean percentage of parasitemia and the percentage of suppression for each group at four days. The best antiplasmodial compounds, **7b** and **8b**, showed a significant activity ($P \leq 0.05$) and a high mean survival rate of about 22 days for mice (Table 1, Figure 3D). More important, compounds **1** and **2** showed 63.71 and 82.27% growth inhibition in a dose of 12.8 mg/kg, whereas compound **4** presented 92.82% at a dose of 25.6 mg/kg. Antiplasmodial activity of these compounds was evaluated using Peter's 4-day suppressive test against inoculated mouse with 1×10^7 *P. berghei*-infected erythrocytes. The compound **8b** indicated 88.32% *in vivo* suppression of parasitemia at the low dosage of 15 mg/kg/day by IP route against inoculated mouse with 2×10^7 *P. berghei*-infected erythrocytes. Therefore, we can draw the conclusion that the compound **8a** is a better candidate than the previously reported compounds.

Lipophilicity plays an important role in biological activity. In the current study, the amino-alcohol compound **7b** with logP= 3.18 showed 90.58% suppression in the high dose (150 mg/kg/day) in comparison to its analog (**7a**, logP= 1.73) that indicated 53.47% suppression in the same dose (Table 1).

However, amino-ether compound **8b** with high lipophilicity (logP= 4.28) was toxic in the concentration higher than 20 mg/kg/day. On the other hand, this compound showed a high suppressive effect in the concentration of 15 mg/kg/day, as compared to its analog **8a** (logP= 3.83; Table 1). Both compounds **7b** with *N,N*-(dibutylamino)propylamino moiety and compound **8b** with *N,N*-dipropylbutan-1-amine moiety presented a high antiplasmodial activity in their groups.

Mechanistic studies have shown that CQ and its analogs interfere with the mechanism of heme polymerization by malaria parasite (25-27). Indeed, in the *P. falciparum* food vacuole (FV) is changed heme to hemozoin, which is a safe pigment for the parasite. This process is essential for the survival of the malaria parasite (26), whereas the antiplasmodial drugs (quinoline analogs) inhibit heme polymerization, which results in accumulation of toxic-free heme in FV and also leads to parasite's death. Therefore, the inhibition of hemozoin formation is an excellent drug target for the development of antimalarial drugs (28). It is assumed that our synthetic compounds can also accumulate in FV and trap in its acidic (protonated) form. As a result, the new compounds have the ability to inhibit the formation of hemozoin and to increase the intracellular heme, which is toxic to the parasite. On the other hand, these compounds with new substitutions at position 4 of phenanthroline ring can have metalloprotease inhibitory activity because of free nitrogen atoms. These mechanistic studies can be evaluated in the next projects of our research group.

Conclusion

The present study illustrates the synthesis of new antiplasmodial compounds with phenanthroline scaffold. The results of this investigation revealed that the best compounds against *P. berghei* were derivatives of amino-alcohol phenanthroline **7b** and amino-ether phenanthroline **8b**. Although the decrease in the percentage of parasitemia was less than the reference drug in infected mice, with the spread of CQ resistance in different regions of the world, the necessity for a new, safe, well-tolerated and an affordable alternative drug is highly felt. Moreover, further research is required to be carried out on these compounds to optimize their antiplasmodial activities such as formulation strategies, co-formulation with other antimalarial drugs, and drug delivery systems.

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Compliance with ethical standards

All applicable and acceptable guidelines for the care and use of animals were considered.

Conflicts of interest

The authors declare no conflicts of interest.

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