

Antiplasmodial Activity and Acute Toxicity of *N*-alkyl and *N*-benzyl-1,10-Phenanthroline Derivatives in Mouse Malaria Model

Mahardika Agus Wijayanti,^{*,a}
Eti Nurwening Sholikhah,^b Iqmal Tahir,^c
Ruslin Hadanu,^c Jumina,^c Supargiyono,^a
and Mustofa^b

^aDepartment of Parasitology Faculty of Medicine, ^bDepartment of Pharmacology and Toxicology Faculty of Medicine, and ^cDepartment of Chemistry Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta-Indonesia, Indonesia

(Received April 13, 2006; Accepted July 27, 2006)

Previous study on *in vitro* antiplasmodial activity of diaza phenanthrene analogs indicated that the 1,10-phenanthroline skeleton represents a potential antimalarial leader compound. Based on those skeletons, six derivatives of *N*-alkyl and *N*-benzyl-1,10-phenanthroline were synthesized and the *in vitro* antiplasmodial activities was evaluated. This paper reported the *in vivo* antiplasmodial activity study of the 1,10-phenanthroline derivatives performed by the classical 4-day suppressive test against *Plasmodium berghei*. Acute toxicity of each compound was determined after a single injection of the compound intraperitoneally in Swiss mice. The 50% effective dose (ED₅₀) of the compound ranged from 2.08 to 50.93 mg/kg of body weight, and the therapeutic indices (TIs) ranged from 2.06 to 7.57 except (1)-*N*-benzyl-1,10-phenanthroline iodide, which was 58.38. All of the 1,10-phenanthroline derivatives had *in vivo* antiplasmodial activity and (1)-*N*-benzyl-1,10-phenanthroline iodide was the most potent.

Key words — 1,10-phenanthroline, antiplasmodial, acute toxicity, therapeutic index

INTRODUCTION

Malaria is the most important parasitic disease of subtropical and tropical countries, and it is constantly changing, especially through the development of parasite resistance to standard antimalarial drugs such as chloroquine. Chloroquine has been the mainstay of falciparum malaria chemotherapy for decades: it is cheap, safe and practicable for outpatient use. Unfortunately, chloroquine resistance is now extensively spread in some countries, including Indonesia. Chloroquine resistance has been widespread at some endemic areas in Indonesia and the percentage of resistance varies from 10–97%. However, chloroquine remains the most commonly used first-line antimalarial drug in Indonesia. Based on data from the Indonesian ministry of health office in 2003, the annual parasite incidence in Java-Bali was 0,22 per 1000 population while the annual malaria incidence in outer Java-Bali was 21,8 per 1000 population.^{1–3)}

Halofantrine has been identified as an effective drug against chloroquine resistant-*P. falciparum*. However this compound is remarkably expensive, and there is no parenteral formulation. In addition this compound is incompletely absorbed via the gastrointestinal tract and that the bioavailability varies. Halofantrine has also reported to prolong the electrocardiographic PR and corrected QT intervals. QT prolongation is a risk factor for ventricular arrhythmias in patients consuming halofantrine.¹⁾

Based on the disadvantages of halofantrine, Yapi *et al.*⁴⁾ have synthesized diaza-analogs of phenanthrene by substituting the two nitrogen atoms in the phenanthrene skeleton. *In vitro* antiplasmodial activity of a series of diaza-analogs of phenanthrene derived from 3-amino, 5-amino, 6-amino, 8-aminoquinoline and 5-aminoisoquinoline showed that among the molecules evaluated the 1,10-phenanthroline skeleton was the most active compound *in vitro* on both chloroquine-resistant (FcB1) and chloroquine-sensitive (Nigerian) strain with an IC₅₀ of about 0.13 μM. Based on the skeleton, Mustofa *et al.*⁴⁾ have also synthesized thirteen derivatives of 1,10-phenanthroline and evaluated the *in vitro* antiplasmodial activity and their structure-activity relationship. Based on the best structure-activity relationship, six new compounds were synthesized: (1)-*N*-methyl-phenanthroline sulfate, (1)-*N*-ethyl-phenanthroline sulfate, (1)-*N*-benzyl-1,10-phenanthroline chloride, (1)-*N*-benzyl-1,10-phenanthroline bromide, (1)-*N*-benzyl-1,10-

*To whom correspondence should be addressed: Department of Parasitology Faculty of Medicine, Gadjah Mada University, Yogyakarta-Indonesia, Indonesia. Tel.: +62-274-6492488; Fax: +62-274-581876; E-mail: dikafkugm@yahoo.co.id

phenanthroline iodide and (1)-*N*-(4-methoxy-benzyl)-1,10-phenanthroline chloride.

The *in vitro* antiplasmodial activity among those compounds showed that five derivatives were active against *P. falciparum* FCR3 and D10 strains with an IC_{50} about 0.10–0.86 μ M, while the IC_{50} of (1)-*N*-(4-methoxy-benzyl)-1,10-phenanthroline chloride was 0.23–3.60 μ M.⁵⁾ This study was aimed to investigate the *in vivo* antiplasmodial activity of the *N*-alkyl- and *N*-benzyl-1,10-phenanthroline derivatives against *P. berghei* and the acute toxicity of the compound in experimental Swiss mice.

MATERIALS AND METHODS

Molecules Tested — Six derivatives of 1-10-phenanthroline were evaluated for their *in vivo* antiplasmodial activity and acute toxicity. The molecules were synthesized by Mustofa *et al.*⁶⁾ Each molecule was different at the substituent on nitrogen atom in position 1 of the 1-10-phenanthroline skeleton. *N*-alkyl-1,10-phenanthroline derivatives

consist of (1)-*N*-methyl-phenanthroline sulfate and (1)-*N*-ethyl-phenanthroline sulfate while *N*-benzyl-1,10-phenanthroline derivatives consist of (1)-*N*-benzyl-1,10-phenanthroline chloride, (1)-*N*-benzyl-1,10-phenanthroline bromide, (1)-*N*-benzyl-1,10-phenanthroline iodide and (1)-*N*-(4-methoxy-benzyl)-1,10-phenanthroline chloride (Fig. 1).

Parasite — For testing the antiplasmodial activity of 1-10-phenanthroline derivatives, Swiss mice were infected with *P. berghei* (ANKA) obtained from Department of Parasitology Faculty of Medicine Gadjah Mada University, Indonesia.

***In vivo* Antiplasmodial Assay** — *in Vivo* antiplasmodial activity of the compound was determined by the classical 4-days suppressive test of Peters *et al.*⁷⁾ Six groups of 10 Swiss mice (5 male and 5 female) weighing about 25 g bred at Department of Pharmacology and Toxicology Faculty of Medicine Gadjah Mada University were used. Each mouse was inoculated with 0.2 ml of 10^7 *P. berghei* infected erythrocytes intraperitoneally on day 0. Mice on group 1 to 6 were treated intraperitoneally

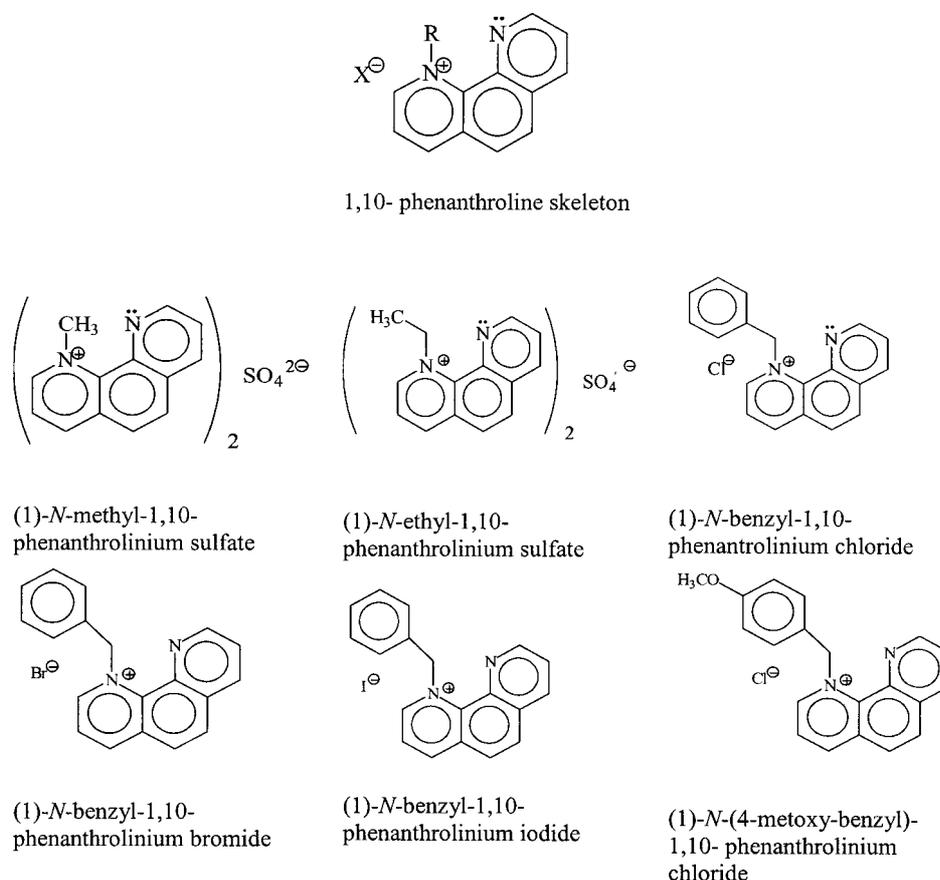


Fig. 1. *N*-alkyl and *N*-benzyl-1,10-phenanthroline Derivatives

Table 1. Parasite Growth Inhibition after 4 days of Treatment with 1,10-Phenanthroline Derivatives on *P. berghei*-Infected Mice

Dose (mg/kg)	Mean \pm S.D. parasite growth inhibition (%)					
	1,10-phenanthroline derivatives					
	(1)- <i>N</i> -methyl-	(1)- <i>N</i> -ethyl-	(1)- <i>N</i> -benzyl- chloride	(1)- <i>N</i> -benzyl- bromide	(1)- <i>N</i> -benzyl- iodide	(1)- <i>N</i> -(4- methoxy-benzyl)-
0.8	ND	ND	ND	ND	28.99 \pm 16.03	22.69 \pm 5.25
1.6	31.33 \pm 7.44	10.88 \pm 6.07	19.52 \pm 9.34	26.88 \pm 7.52	55.01 \pm 2.42	39.42 \pm 23.04
3.2	49.67 \pm 11.88	23.99 \pm 3.57	65.43 \pm 18.94	40.65 \pm 3.59	69.94 \pm 19.82	38.03 \pm 19.99
6.4	20.85 \pm 14.45	9.72 \pm 3.24	42.09 \pm 23.31	16.69 \pm 12.56	43.06 \pm 25.19	29.42 \pm 15.96
12.8	27.34 \pm 9.71	28.16 \pm 5.25	ND	63.71 \pm 18.36	82.27 \pm 14.48	45.94 \pm 23.47
25.6	92.82 \pm 4.21	46.68 \pm 18.73	ND	ND	ND	ND

ND: Not determined.

with 0, 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 mg/kg of the compound respectively perform on the day of infection and repeated on day 1, day 2, and day 3 after the day of infection. Parasitemia levels were determined on the day following the last treatment. The ED₅₀ which is the dose leading to 50% parasite growth inhibition compared to growth in the negative control (treated with an equal volume of normal saline), was evaluated from a plot of activity (expressed as a percentage of the activity in the controls) versus the log dose.

Assesment of Acute Toxicity in Mice — Acute toxicity of each drug was evaluated after a single intraperitoneal injection of drug to fifty randomly bred male and female Swiss mice weighing 30 to 40 g with four drug doses divided into 5 groups. The drugs were administered intraperitoneally in a volume of 0.2 ml. The LD₅₀ was expressed as the 50% lethal dose, which corresponds to the dose leading to 50% deaths 14 days after drug injection. These experiments were conducted in accordance with the experimental animal guidelines of laboratory method for toxicology at Gadjah Mada University and the guidance of clinical testing for traditional medicine at the Indonesian Ministry of Health.^{8,9)}

RESULTS

Using the 4-days suppressive test on mice infected with *P. berghei* we could observe a substantial reduction of parasitemia compared to control, untreated mice. After 4 days of treatment with 1,10-phenanthroline derivatives, mean inhibition of parasite growth in the *P. berghei*-infected mice ranged from 10.88 \pm 6.07% to 92.82 \pm 4.21% (Table 1) and the mean inhibition of parasite growth in the chlo-

Table 2. Parasite Growth Inhibition after 4 days of Treatment with Chloroquine on *P. berghei*-Infected Mice

Dose (mg/kg)	Mean \pm S.D. parasite growth inhibition (%)
0.04	56.49 \pm 20.14
0.13	40.99 \pm 32.76
0.40	25.49 \pm 21.12
1.20	57.79 \pm 28.16
3.60	99.29 \pm 0.74

roquine-positive control group ranged from 25.49 \pm 21.12% to 99.29 \pm 0.74% (Table 2). The reduction of parasitemia was never complete, although in (1)-*N*-methyl-1,10 phenanthroline sulfate achieved a maximal inhibition of 92.82%. In this infection the ED₅₀ for *N*-alkyl- and *N*-benzyl-1,10-phenanthroline derivatives were ranged from 2.08 mg/kg to 50.93 mg/kg while the ED₅₀ of chloroquine was 0.20 mg/kg (Table 3).

The 1,10-phenanthroline derivatives were also tested for their acute toxicities after intraperitoneal injection of Swiss mice. Their LD₅₀s ranged from 25.94 to 132.89 mg/kg of body weight. Some compounds such as (1)-*N*-methyl-phenanthroline sulfate, (1)-*N*-ethyl-phenanthroline sulfate and (1)-*N*-benzyl-1,10-phenanthroline iodide have LD₅₀s greater than chloroquine with an LD₅₀ 69.57 mg/kg. Therapeutic indices (TIs) were calculated on the basis of acute LD₅₀/ED₅₀ ratio. For most of the 1,10-phenanthroline derivatives, *i.e.*, (1)-*N*-methyl-phenanthroline sulfate, (1)-*N*-ethyl-phenanthroline sulfate, (1)-*N*-benzyl-1,10-phenanthroline chloride and (1)-*N*-benzyl-1,10-phenanthroline bromide, the TI achieved in the intraperitoneal mode ranged from 2.06 to 7.57 while

Table 3. Antiplasmodial Activities and Toxicities of Various 1,10-Phenanthroline Derivatives

1,10-phenanthroline derivatives	LD ₅₀ (mg/kg)	ED ₅₀ (mg/kg)	Therapeutic Index
	In mice		
	Acute	<i>P. berghei</i>	
(1)- <i>N</i> -methyl-1,10-phen-sulfate	70.05	9.34	7.50
(1)- <i>N</i> -ethyl-1,10-phen-sulfate	132.89	50.93	2.61
(1)- <i>N</i> -benzyl-1,10-phen-chloride	38.72	5.11	7.57
(1)- <i>N</i> -benzyl-1,10-phen-bromide	25.94	12.57	2.06
(1)- <i>N</i> -benzyl-1,10-phen-iodide	121.42	2.08	58.38
(1)- <i>N</i> -(4-methoxy benzyl)-1,10-phen-chloride	ND	47.27	ND
Chloroquine	69.57	0.20	347.85

Acute LD₅₀s were determined for mice by intraperitoneal administration. ED₅₀s were determined by a 4-day suppressive test with *P. berghei* infected mice. The TI is the LD₅₀/ED₅₀ ratio and is based on acute toxicity. ND: Not determined.

the TI of (1)-*N*-benzyl-1,10-phenanthroline iodide was 58.38 (Table 3).

DISCUSSION

We investigated the antimalarial potencies of *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives in *P. berghei* infected mice. Treatment with those compounds significantly inhibited parasitemia compared to the non-treated group. Although the suppression of parasitemias was never complete (100% inhibition of parasite growth), the results indicate antiplasmodial potential.

Drugs act on organism by specifically interfering with cellular or biochemical processes (targets). The classic example of a drug target is an enzyme that's production may be inhibited by modifying its active sites to reduce its activity on its substrate and others. Effective drugs may exhibit a selective toxicity for the pathogen as compared to the host cells. Many factors contribute to this selective toxicity and these factors are not mutually exclusive. Rational drug design seeks to exploit these various factors to develop drugs which are highly toxic to the pathogen and at the same time exhibit minimal toxicity to the host.⁹⁾

Relatively few new antimalarial drugs are undergoing clinical testing. Halofantrine, identified in the 1940s, was not developed until the 1980s, and its use has been limited by variable oral absorption and cardiac toxicity. The drug is approved in the United States for treatment of chloroquine-resistant *P. falciparum* infection.^{5,10)} Halofantrine is a non-natural antimalarial agent possessing a phenanthrolic skeleton, which has been reported to have good

therapeutic effect but with some important side effects.⁴⁾ As the 1,10-phenanthroline ring system is a well-known metalloprotease inhibitor by chelating divalent metal ions,⁴⁾ we can reasonably also suppose that this phenomenon is implicated in the mechanism of action against malaria parasite.

Antimalarial drug development can follow several strategies, ranging from minor modification of existing agents to the design of novel agents that act against new target. Development of analogs of existing agents by chemical modifications of the compounds is to improve upon existing antimalarial agents. For example, chloroquine, primaquine and mefloquine were discovered through chemical strategies to improve upon quinine. An 8-aminoquinoline, tafenoquine, offer improved activity against hepatic-stage parasites over that of the parent compound, primaquine, and is effective for antimalarial chemoprophylaxis. Since halofantrine use is limited by toxicity, the analog lumefantrine was developed and is now a component of the new combination co-artemether.¹¹⁾ Structure modification of mefloquine has been established by Karle and Karle.¹²⁾ (-)-Mefloquine has the same stereochemistry as quinine, and (+)-mefloquine has the same stereochemistry as quinidine. Since quinine and quinidine possess different antimalarial activities, the (+) and (-) isomers of mefloquine were tested against *P. falciparum* *in vitro*. (+)-Mefloquine is more potent than (-)-mefloquine *in vitro* against the D6 and W2 strains of *P. falciparum* and quinidine is more potent than quinine. Antimalarial mechanism and the relationship between the physicochemical properties and the antimalarial activities of 23 artemisinin analogues have been evaluated by Cheng *et al.*¹³⁾ Artemisinin is an effective antimalarial agent against

chloroquine-resistant *P. falciparum* strains and cerebral malaria. However its poor solubility restricts its employment. In order to find more potent anti-malarial derivatives a series of C12 ether and ester analogues have been synthesized. The study showed that introduction of groups to the 12-position of the dihydroartemisinin can improve their solubility.

Modification of drug structure is one possible method to get higher activity and lower toxicity while still acting on similar targets. This study showed that substitution of varies functional group in (1)-*N*-1,10-phenanthroline change its antiplasmodial activity. Activity of *N*-benzyl-1,10-phenanthroline derivatives was higher than that of *N*-alkyl-1,10-phenanthroline derivatives. *N*-benzyl-1,10-phenanthroline derivatives had greater proportion of non-polar molecules. This condition may support the compound to penetrate the cell membrane of micro-organism, therefore, giving it higher antimicrobial activity.¹⁴⁾

Based on the structure, (1)-*N*-benzyl-1,10-phenanthroline chloride, (1)-*N*-benzyl-1,10-phenanthroline bromide and (1)-*N*-benzyl-1,10-phenanthroline iodide are non polar compared to (1)-*N*-methyl-1,10-phenanthroline sulfate and (1)-*N*-ethyl-1,10-phenanthroline sulfate due to the existence of benzyl moieties in the former of structure. When compared to (1)-*N*-benzyl-1,10-phenanthroline chloride which exists as a chloride salt, (1)-*N*-benzyl-1,10-phenanthroline iodide should be more effective to interact with the cell membranes as this compound posses softer anion conjugate (I⁻). Likewise, the antimalarial activities of (1)-*N*-(4-methoxy-benzyl)-1,10-phenanthroline chloride was lower than those (1)-*N*-benzyl-1,10-phenanthroline bromide and (1)-*N*-benzyl-1,10-phenanthroline iodide as the molecular size of that compound is perhaps too bulky for the occurrence of an effective interaction with the cell membranes.

The 4-aminoquinolines, chloroquine and amodiaquine, the quinoline methanols, quinine and mefloquine, and the phenanthrene methanol, halofantrine all exert selective toxicity towards the erythrocytic stages of malaria parasites and were developed based on a knowledge of quinine structure and activity. Morphological effects following treatment with mefloquine, quinine and halofantrine are similar to those observed following treatment with chloroquine, *i.e.* an initial swelling of the acid food vacuole. It is accepted that these antimalarial agents exert their antimalarial effects by interacting with the hemoglobin degradation process within the

parasite, probably through an interaction with hemozoin, although the absolute mechanism of action is still debated.^{10,15)}

Further study is planned to evaluate the mechanism of action of the most active compound *i.e.* haem polymerization inhibition assay, protease inhibition assay and KCl-sodium dodecyl sulfate (SDS) precipitation assay. Study of the pharmacokinetics on animal model of these compound have also been planned.

In conclusion, we have reported the *in vivo* antiplasmodial activity and acute toxicity of *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives on *P. berghei*-infected mice. From this evaluation those compounds appeared as potential antimalarial agents. Among the six 1,10-phenanthroline derivatives, 1-*N*-benzyl-1,10-phenanthroline iodide had the most effective antiplasmodial activity.

Acknowledgements The study was funded by Integrated Excellent Research from Ministry of Research & Technology and Postgraduate Research Grant from Ministry of National Education, Indonesian Government. We are grateful to PT Konimex Indonesia for providing the chloroquine diphosphate used in these test. Thanks also to the Department of English Faculty of Cultural Science GMU for English editing support.

REFERENCES

- 1) Winstanley, P. A. (2000) Chemotherapy for Falciparum malaria: The Armoury, the Problems and the Prospects. *Parasitol. Today.*, **16**,146–153.
- 2) Franssen, F. F. J., Smeijsters, L. J. J. W., Beger, I. and Aldana, B. E. M. (1997) *In vivo* and *in vitro* antiplasmodial activities of some plants traditionally used in Guatemala against malaria. *Antimicrob. Agents Chemother.*, **41**,1500–1503.
- 3) Tropical Diseases Center (2004) *Symposium of malaria control in Indonesia*, TDC, Airlangga University Surabaya, Indonesia.
- 4) Yapi, A. D., Mustofa, M., Valentin, A., Chavignon, O., Teulade, J., Mallie, M., Chapat, J. and Blace, Y. (2000) New Potential antimalaria agents : synthesis and biological activities of original diaza-analogs of phenanthrene. *Chem. Pharm. Bull.*, **48**, 1886–1889.
- 5) Mustofa, M., Yapi, A. D., Valentin, A. and Tahir, I. (2003) *In vitro* antiplasmodial activity of 1,10-phenanthroline derivatives and its quantitative structure-activity relationship. *BKed.*, **35**, 67–74.

- 6) Mustofa, M., Jumina, J., Wijayanti, M. A., Tahir, I. and Sholikhah, E. N. (2005) Developing 1,10-phenanthroline derivatives as New Antimalaria agents. In *Report of 10th Integrated Excellent Research from Ministry of Research & Technology Indonesian Government*.
- 7) Peters, W. J., Portus, J. H. and Robinson, B. L. (1975) The chemotherapy of rodent malaria XXII. The value of drug-resistant strains of *P. berghei* in screening for blood schizonticidal activity. *Ann. Trop. Med. Parasitol.*, **69**, 155–171.
- 8) Ngatidjan, D. (1991) *Laboratory method for toxicology*, Gadjah Mada University.
- 9) Dirjen POM Indonesian Ministry of Health (2000) *Guidance of clinical testing for traditional medicine*.
- 10) Rosenthal, P. J. (1998) Proteases of malaria Parasites : New Targets for Chemotherapy. *J. Emerg. Inf. Diseases.*, **4**, 49–57.
- 11) Rosenthal, P. J. (2003) Review Antimalarial drug discovery: old and new approaches. *J. Exp. Biol.*, **206**, 3735–3744.
- 12) Karle, J. M. and Karle, I. L. (2002) Crystal structure of (–) mefloquine hydrochloride reveals consistency of configuration with biological activity. *Antimicrob. Agents Chemother.*, **46**, 1529–1534.
- 13) Cheng, F., Shen, J., Luo, X., Zhu, W., Gu, J., Ji, R., Jiang, H. and Chen, K. (2002) Molecular docking and 3-D-QSAR studies on the possible antimalarial mechanism of artemisinin analogues. *Bioorg. Med. Chem.*, **10**, 2883–2891.
- 14) Siswandono, Soekardjo, B. (2000) *Medicinal Chemistry-I*, Airlangga university Press, Indonesia.
- 15) Mungthin M., Bray, P. G., Ridley, R. G. and Ward, S. A. (1998) Central role of hemoglobin degradation in mechanisms of action of 4-aminoquinolines, quinoline methanols, and phenanthrene methanols. *Antimicrob. Agents Chemother.*, **42**, 2973–2977.