SYNTHESIS AND ANTIPLASMODIAL ACTIVITY TESTING OF (1)-*N*-(4-METHOXYBENZYL)-1,10-PHENANTHROLINIUM BROMIDE

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ABSTRACT

Synthesis of (1)-N-(4-methoxybenzyl)-1,10-phenanthroline bromide from 1,10-phenanthroline monohydrate and 4-methoxybenzaldehyde as starting material and evaluation of its antiplasmodial activities have been carried out. The 4-methoxybenzyl alcohol was prepared from 4-methoxy-benzaldehyde using sodium borohydride ($NaBH_4$) reagent and ethanol absolute solution. The mixture was refluxed for 3 h. To yield colorless dilution compound with 90.41 % in efficiency. Furthermore, bromination of 4-methoxybenzyl alcohol with phosphorus bromide (PBr_3) was conducted by refluxing for 3 h. The product of this reaction was yellow liquid of 4-methoxybenzyl bromide, 79.03% yield and 95.34 % purity. The final step of reaction was benzylation of 1,10-phenanthroline monohydrate with 4-methoxybenzyl bromide reagent. It was conducted by refluxing in aceton for 8 h at 55 ^oC. The yield of the reaction was (1)-N-(4-methoxybenzyl)-1,10-phenanthroline bromide (77.63%). It is pink solid form, and its melting point is 192-193 ^oC. Identification of the product was carried out by means of GC-MS, IR and ^1H-NMR spectrometers. The in vitro antiplasmodial activity on chloroquine-resistant Plasmodium falciparum *FCR-3 strain and chloroquine sensitive* P. falciparum *D10 strain for (1)-N-(4-methoxybenzyl)-1,10-phenanthroline bromide were determined by microscopic method. The result showed that after 72 h incubation, it has IC_{50} 0.93±0.02 µM and 1.21±0.09 µM, respectively.*

Keywords: *1,10-phenanthroline, (1)-N-(4-methoxybenzyl)-1,10-phenanthroline bromide, 4 methoxybenzaldehyde, antiplasmodial activities*

INTRODUCTION

Malaria, a tropical disease caused by protozoan parasites of the genus *Plasmodium,* has been a real concern for centuries and is now extended to more than 40% of the world's population. *Plasmodium falciparum,* the most prevalent species across the globe, may cause cerebral malaria that is often fatal [1]. Word Health Organization (WHO) estimated that, in 2006, there were 300-500 million cases of malaria and more than 1.5-2.7 million deaths due to it. Furthermore, international travel becomes more common. For this reason, malaria is not confined to the tropical zones of the world, and imported malaria is an increasingly serious problem.

Chemotherapy remains as one of the most rational measures to control the intolerable burden of malaria as antimalarial vaccine is not yet available. Beside it is proven that the vector control measures are very difficult to sustain in most endemic setting. However, the rapid spread of the malarial parasite resistance to the antimalarial drug mainstay, chloroquine (CQ) and sulfadoxine-phyremethamine, within the last few decades alerted to the efforts to develop alternative antimalarial drugs. Chloroquine is a 4-aminoquionoline derivative antimalarial drug that was introduced near the end of World War II, and remains as chosen the drug for vivax malaria in many parts of the world. Its therapeutic efficacy and safety, wide distribution, ready availability and relatively low price quickly proved this drug to be one of the most successful and important drugs ever deployed against an infectious disease. Its heavy use in subsequent decades has however led to CQ resistance in *P. falciparum* at the end of 1950s and has spread rapidly throughout the world afterwards [2].

The halofantrine as new antimalarial has good therapeutic effects [3]. Halofantrine is more active against strains of *P. falciparum* that are resistant to chloroquine, pyrimethamine, and quine [4]. However, halofantrine is known to have some unwanted side effects, such as abdominal pain, nausea, vomiting, diarrhea, orthostatic, hypertension, prolongation of QTc intervals, pruritus, rash [5] and hepatotoxic [6]. The 1,10-phenanthroline derivatives are similar to halofantrine as antimalarial drug which its added at heterocyclic with two nitrogen atoms. In 2000, Yapi reported that the 1,10-phenanthroline ring system appeared as a new class of potential antimalarial compound [7]. Now, as part of our research concerning the synthesis and biological activities of 1,10-phenanthroline derivatives. Mustofa was synthesized

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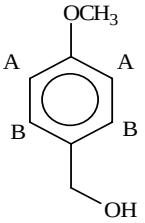
thirteen derivatives of 1,10-phenanthroline and evaluated their *in vitro* antiplasmodial activities and Quantitative Structure-Activity Relationship (QSAR) [8]. Based on the QSAR model of the 1,10-phenanthroline derivatives, 12 new compound of *N*-alkyl-and *N*-benzyl 1,10-phenanthroline derivatives were synthesized and were evaluated for their *in vitro* and *in vivo* antiplasmodial activity [9-17]. This study was conducted to synthesize a new derivate of *N*-benzyl-1,10-phenanthroline i.e. (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide and to evaluate its *in vitro* antiplasmodial activity.

EXPERIMENTAL SECTION

In general, the melting points of compound were determined on melting point electro thermal 9100 and are not corrected. The spectrum of structures compound measurements was taken using the following instruments: FTIR spectrums were taken on Shimadzu FTIR-8201 PC; ^{1}H-NMR spectrums were obtained on JEOL 60 MHz and JEOL 500 MHz. MS spectrum was recorded on GC-MS Shimadzu QP 5000.

Synthesis of 4-methoxybenzyl alcohol (2)

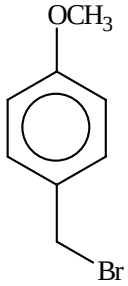


A mixture of powdered sodium borohydride (4.52 g, 0.12 mol) and ethanol absolute (60 mL) was stirred at room temperature for 20 min, then 4-methoxybenzaldehyde **(1)** (5.44g, 0.04 mol) was added, and the mixture was refluxed for another 3 hours. The result of reaction was evaporated to remove ethanol, and the mixture was diluted with water (50 mL), neutraled with HCl 11%. The mixture was extracted with ethyl acetate (3x25 mL), and the combined organic layers were washed with water. After it was washed with water, the mixture was dried over sodium sulfate anhydrous and evaporated to get colorless oil (4.49 g, 90.41%). Found: FT-IR (net): 3348.2 cm^{-1}, 3001.0 cm^{-1}, 2935.5 cm^{-1}, 2873.7 cm^{-1}, 1612.6 cm^{-1}, 1585.4 cm^{-1}, 1512.1 cm^{-1}, 1461.9 cm^{-1}, 1450.0 cm^{-1}, 1373.2 cm^{-1}, 1176.5 cm^{-1}, 1110.9 cm^{-1}, and 817.8 cm^{-1}. ^{1}H-NMR ($CDCl_3$; 60 MHz) δ: 7.3-7.0 (2H, d, H_A), 6.9-6.7 (2H, d, H_B), 4.2 (2H, s, H_{CH2}), 4.0 (1H, s, H_{OH}), and 3.7 (3H, s, H_{CH3}).

Synthesis of phosporus tribromide as reagent

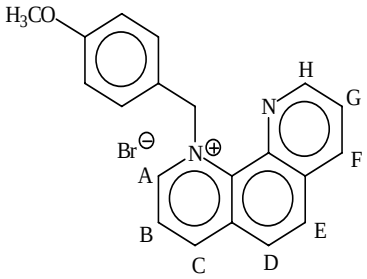
A powdered of phosphorus red (18.6 g, 0.60 mol) in carbon tetrachloride (135 mL) was stirred in room temperature for 1h. The liquid of bromine (132.24 g, 0.83 mol) was dropped slowly and stirred continuously for another 1 h. The mixture was refluxed 1h, then the result of reaction was distillated to give two fractions i.e. carbon tetrachloride as solvent (bp 71-72 ^{o}C) and phosphorus tribromide as product of reaction (bp 172-173 ^{o}C, bp theoretical 175 ^{o}C).

Synthesis of 4-methoxybenzyl bromide (3)



A 4-methoxybenzyl alcohol **(2)** (2.47 g, 0.018 mol) was added into a solvent carbon tetrachloride (40 mL). The mixture was stirred at room temperature, and then phosphorus tribromide (4.8 mL PBr_3 in 5.2 mL of carbon tetrachloride) was added slowly into a mixture, drop by drop. The mixture was continuously stirred to be continue at room temperature for 30 min, then at 50-60 ^{o}C for another 2 h. The result of reaction was diluted with solution of sodium bicarbonate (5%, 2x20 mL) and then washed with water (2x30 mL). The combinating of organic layers was dried over sodium sulfate anhydrous and evaporated to get yellow oil (2.98g, 79.13%). Found: FT-IR (net): 3004.9 cm^{-1}, 2935.5 cm^{-1}, 2835.2 cm^{-1}, 1608.5 cm^{-1}, 1512.1 cm^{-1}, 1461.9 cm^{-1}, 1370.0 cm^{-1}, 1253.6 cm^{-1}, 1176.5 cm^{-1}, 1099.5 cm^{-1}, 1033.8 cm^{-1}, 833.2 cm^{-1}, and 759.9 cm^{-1}. GC-MS (purity 95.34%) (relative intensity) *m/z*: 202 [M$^+$]; 202, 122, 121 (base peak), 106, 91, 78 and 51.

Synthesis of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide (5)



A solution of 1,10-phenanthroline (4) (0.39 g, 0.002 mol) and 4-methoxybenzyl bromide **(3)** (1.0 g, 0.005 mol) in acetone (25 mL) was refluxed for 8 hours. The mixture of result reaction was cooled. The precipitate which formed was filtered, and washed with acetone. Yield: FT-IR (KBr): 3340.5 cm^{-1}, 3028.0 cm^{-1}, 2993.3 cm^{-1}, 1596.9 cm^{-1}, 1542,9 cm^{-1}, 1469.7 cm^{-1}, 1454.2 cm^{-1}, 1364.0 cm^{-1}, 1226.2 cm^{-1}, 1033.8 cm^{-1},

883.5 cm^{-1}, 848.6 cm^{-1}, 717.3 cm^{-1}, and 621.0 cm^{-1}. ^{1}H-NMR (DMSO-d6; 500 MHz) δ: 9.18-9.11 (1H, s, H_A), 8.93-8.92 (1H, s, H_C), 8.66-8.61 (1H, s, H_H), 8.24-8.23 (1H, s, H_B); 8.05-8.03 (1H, d, H_F), 7.98 (1H, m, $H_{E\&D}$), 7.00 (2H, s, H_{CH2}), 6.90-6.82 (4H, m, H_{ph}), 4.41-4.36 ($H_{hidrogen\ bonding}$) and. 3.31 (3H, s, H_{CH3}).

BIOLOGICAL ACTIVITY

Parasites were cultured according to the method described by Trager and Jensen [18] and modified by Benoit [19]. FCR-3 was considered as a chloroquino resistant strain and D10 was considered as a chloroquine sensitive strain. The culture medium was replaced daily and the cultures were synchronized by 5% D-Sorbitol lysis before using (Merk, Darmstadt, Germany). The method used for *in vitro* antimalarial activity testing was adapted from microscopic method. The molecules were tested 3 times in triplicate in 96-well plates (Nunc, Denmark) with plasmodium at ring stage at 2% parasitemia, 3% hematocrit. For each test, the parasite cultures were incubated with molecul tested at various concentrations for 72 h. Parasite growth was estimated by painting with Giemsa (5%) for 30 seconds. The parasite control in the presence without molecul testes was referred to as 100% growth. The concentrations inhibiting 50% of the parasite (IC_{50}) were determined by SPPS 13.0 software. The IC_{50} was used to discribe antiplasmodial activity of the molecul tested.

RESULT AND DISCUSSION

The synthesis of the 1,10-phenanthroline derivatives can be conducted from 8-aminoquinoline and 1,10-phenanthroline as starting materials. In this study, we focused on synthesis 1,10-phenanthroline derivatives from 1,10-phenanthroline and 4-methoxy-benzaldehide as starting materials. There are three steps of the reactions for synthesis (1)-N-(4-methoxybenzyl)-1,10-phenanthrolinium bromide compound. The first step of reaction was reduction of 4-methoxybenzaldehyde to get colorless oil (4-methoxybenzyl alcohol) with 90.41% in efficiency. The second step of process was bromination of 4-methoxybenzyl alcohol with phosphorus tribromide to get yellow oil of 4-methoxybenzyl bromide, 79.03% yield and 95.34% purity. Steps of synthesis mentioned above represented the process of reagen alkylation and then reacted with 1,10-phenanthroline monohydrate **(4)**. The final step reaction was *N*-benzylation of 1,10-phenanthroline monohydrate with 4-methoxybenzyl bromide **(3)** reagent to yield: (1)-*N*-(4-methoxybenzyl)-1,10-phenanthroline bromide (77.63%), pink solid form, melting point 192-193^{o}C, and the scheme reaction is shown in Fig 1.

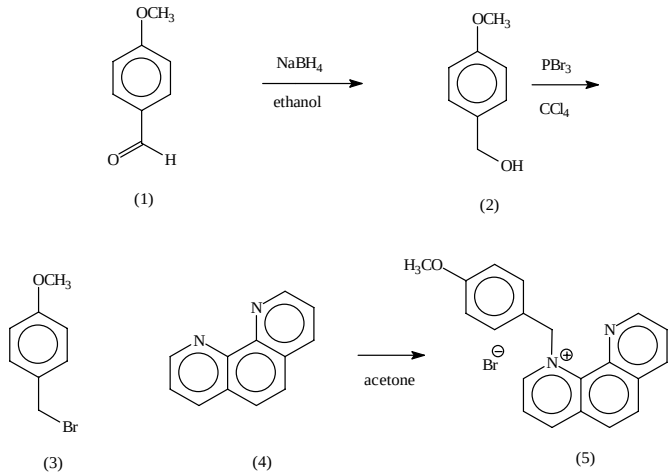


Fig 1. Schema reaction of synthesis (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide compound

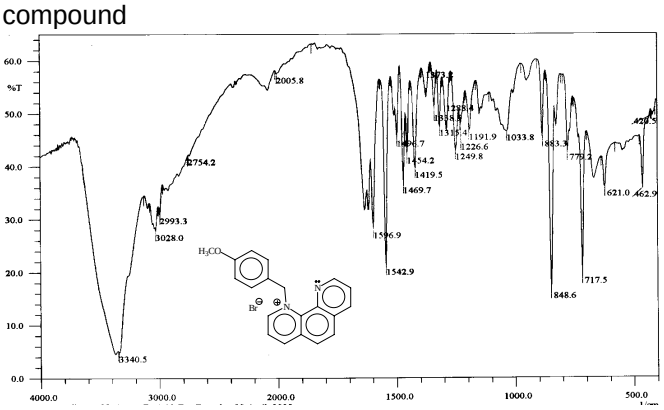


Fig 2. FTIR spectrum of (1)-*N*-(4-methoxybenzyl) 1,10-phenanthrolinium bromide

Structural determination of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide was made on the basis of FTIR and ^{1}H-NMR studies. The FTIR spectrum showed pick in 3340.5 cm^{-1} indicated the product of reaction has hidrogen bonding, and FTIR spectrum is shown in Fig 2.

The product of *N*-benzylation reaction was characterized by ^{1}H-NMR. In the ^{1}H-NMR spectrum of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide as product showed eleven proton species. The chemical shift (δ) at 4.41-4.36 ppm indicated hidrogen bonding between (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide and H_2O, and it agrees with infrared spectrum. The specific spectra showed at δ 7.00 ppm with singlet splitting indicated proton of pick from methylene (-CH_2-). The complete result showed in Fig. 3 and Table 1.

Based on infrared spectrum and ^{1}H-NMR spectrum, the product of *N*-benzylation reaction was indicated of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide **(5)** compound.

Table 1. List of chemical shift of ^{1}H-NMR spectrum from experiment and Chem Office Software

Proton	Experiment Data			Chem Office Data	
	δ (ppm)	Amount Proton	Splitting	δ (ppm)	Estimation Quality
A	9.18-9.11	1	singlet	9.20	red=rough
C	8.93-8.92	1	singlet	9.00	red=rough
H	8.66-8.65	1	singlet	8.81	blue=good
B	8.24-8.23	1	singlet	8.50	red=rough
F	8.05-8.03	1	singlet	8.00	blue=good
E&D	7.98	2	singlet	7.68-7.43	blue=good
G	7.50-7.46	1	singlet	7.26	blue=good
CH_2	7.00	2	singlet	2.60	red=rough
Ph	6.90-6.82	4	multiplet	6.95-6.65	blue=good
CH_3	3.31	3	singlet	3.73	blue=good

Table 2. Parasite Growth Inhibition and IC_{50} of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide on FCR-3 strain

Concentration (ng/mL)	Inhibition (%)			IC_{50} (µM)		
	Rep. I	Rep. II	Rep. III	Rep. I	Rep. II	Rep. III
800	83.12	84.55	82.78			
400	39.77	39.39	38.83	0.82	0.81	0.84
200	35.45	34.33	35.27			
100	31.14	30.76	30.58			
50	14.25	15.19	14.63			
25	8.25	6.94	6.00			
Mean				0.82±0.01		

Rep. = replication.

Table 3. Parasite Growth Inhibition and IC_{50} of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide on D10 strain

Concentration (ng/mL)	Inhibition (%)			IC_{50} (µM)		
	Rep. I	Rep. II	Rep. III	Rep. I	Rep. II	Rep. III
2000	95.48	97.50	93.37			
1000	91.89	91.93	91.55			
500	36.37	39.34	38.29	1.16	1.15	1.32
250	21.88	20.34	14.97			
125	16.98	14.68	8.54			
Mean				1.21±0.09		

Rep. = replication.

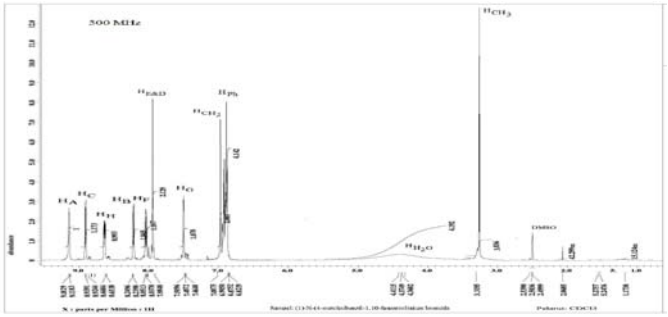


Fig. 3. Proton NMR Spectrum of (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide

The result of investigation antiplasmodial activities using chloroquine-resistant FCR-3 strain is summarized in Table 2. Whereas, the result of evaluation antiplasmodial activities using chloroquine-sensitive D10 strain is summarized in Table 3.

Sholikhah [17] reported the activities of 8 new compounds of *N*-alkyl and *N*-benzyl-1,10-phenanthrolinium derivatives: 1) (1)-*N*-methyl-1,10-penanthrolinium sulfate, 2) (1)-*N*-ethyl-1,10-penanthrolinium sulfate, 3) (1)-*N*-*t*-buthyl-1,10-penanthrolinium chloride, 4) (1)-*N*-benzyl-1,10-penanthrolinium chloride, 5) (1)-*N*-benzyl-1,10-penanthrolinium bromide, 6) (1)-*N*-benzyl-1,10-penanthrolinium iodide, 7) (1)-*N*-(4-methoxybenzyl-1,10-penanthrolinium chloride, and 8) (1)-*N*-(4-benzyloxy-3-methoxybenzyl-1,10-penanthrolinium chloride compounds. Of the 8 compounds tested, the compounds **(5)** and **(6)** had the highest activity (IC_{50}=0.10-0.13µM and 0.18-0.23 µM, respectively), against FCR-3 strain. Compound **(6)** had the highest activity (IC_{50}=0.33-0.34µM) on D10 strain *P. falciparum.*

This treatment with (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide compound significantly inhibited parasitemia of *P. falciparum* FCR-3 strain and D10 strain. Although the suppression of parasitemias was never complete (100% inhibition of parasite growth), the results indicate antiplasmodial potential, but it had lower activity compared to **(5)** and **(6)** compound. The (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide had higher activity than compound (1)-*N*-*t*-buthyl-1,10-penanthrolinium chloride (1.84-7.15µM) and (1)-*N*-(4-benzyloxy-3-methoxybenzyl-1,10-penanthrolinium chloride (1.08-2.19µM).

CONCLUSION

The (1)-*N*-(4-methoxybenzyl)-1,10-phenanthrolinium bromide was synthesized, characterized, and evaluated of *in vitro* antiplasmodial activity. Three steps of synthesis are reduction, bromination and *N*-alkylation and the yields: pink solid form (77.63%), melting point 192-193 ^{o}C. Results of *in vitro* antiplasmodial activity on chloroquine-resistant *Plasmodium falciparum* FCR-3 strain and chloroquine sensitive *P. falciparum* D10 strain were determined by microscopic method after 72 h incubation have IC_{50} 0.93 ± 0.02µM and 1.21 ± 0.09 µM, respectively.

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REFFERENCES

1. Robbert, A., Vical, F.A., Cabaret, O.D., and Meunier, B., 2001, *Pure Appl. Chem.,* 73, (7): 1173-118.
2. Baird, J.K., Widay, I., Fryauff, D.J., Sutanihardja, M.A., Leksana, B., Widjaya, H., Kysdarmanto, and Subianto, B., 1997, *Am J Trop Med Hyg,* 56: 627-631.
3. Basco, L. K., Ruggeri, C., and Le Bras, J., 1994, Molecules Antipaladiques: Relations Structure-Activity, *ed Masson,* 108-234.
4. Rang, H.P., Dale, M.M., Ritter J.M., and Moore P.K., 2003, *Pharmacology*, 5th ed, Edinburgh: Churchill Livingstone.
5. Karbwang, J., and Na-Banchang, K., 1991, *Clin Phamacokinet,* 27: 104-109.
6. Bassi, P.U., Buratai, B.I., and Kuchali, W., 2006, *AJBR,* 9: 31-35.
7. Yapi, A.D., Mustofa, M., Valentin A., Chavignon O., Teulade, J., Mallie, M., Chapat, J., and Blace, Y., 2000, *Chem. Pharm. Bull.,* 48: 1886-1889.
8. Mustofa, M., Yapi, A. D., Valentin, A., and Tahir, I., 2003, *J Med Sci.,* 35: 67-74.
9. Hadanu, R., 2004, *Syntesis of antimalarial (1)-N-alkyl-1,10-phenanthrolinium and 3-(2-hydroxy-ethyl)-2-methyl-1,10-phenanthroline-4-ol,* Thesis, Gadjah Madah University, Yogyakarta.
10. Hadanu, R., Anwar C., Jumina, and Mustofa, 2005, *Journal Science and Sibernatika*, 18 (3): 297-307.
11. Supargiyono, Jumina, and Mustofa, 2005, *New antimalarial of (1)-N-benzil-1,10-phenanthroline: Synthesis, antiplasmodial activity, toxicity, phamacokinetic, action mechanism and their formula.* Research Report of Post Graduate Research Grant. Jakarta: Minister of Education Indonesian Goverment.
12. Mustofa, Jumina, Wijayanti M.A., Tahir, I., and Sholikhah, E.N., 2005, D*evelopment of New Compounds of 1,10-phenanthroline Derivatives as Antimalarial Drug,* Researh Report of 10th Competitive Integrated Research. Jakarta: Minister of Research and Technology Indonesian Goverment.
13. Hadanu, R., Matsjeh S., Jumina, Mustofa, Wijayanti M.A., and Sholikhah E.N., 2006, *Synthesis of antimalarial compound (1)-N-(4-ethoxybenzyl)-1,10-phenanthrolinium chloride,* Proceding of Seminar and Congress Association Chemist of Indonesian, Jakarta.
14. Hadanu, R., Matsjeh S., Jumina, Mustofa, Wijayanti M.A., and Sholikhah E.N., 2006, *Synthesis Of Antimalarial Drug (1)-N-(4-ethoxybenzyl)-1,10-phenanthrolinium Bromide*, Proceding of International Seminar on Department of Chemistry, Gadjah Mada University, Yogyakarta.
15. Hadanu, R., Matsjeh S., Jumina, Mustofa, Wijayanti M.A., and Sholikhah E.N., 2006c, *Synthesis of (1)-N-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride as Antimalarial,* Proceding of Claster Scince-Technology Seminar, Gadjah Madah University, Yogyakarta.
16. Wijayanti, M.A., Sholikhah, E.N., Tahir, I, Hadanu, R., Jumina, Supargiyono, and Mustofa, 2006, *J Health of Sci,* 52 (6): 794-799.
17. Sholikhah, E.N., Supargiyono, Jumina, Wijayanti, M.A., Tahir, I, Hadanu, R., and Mustofa, 2006, *Southeast Asian J Trop Med Public Health,* 37 (6): 072-1077.
18. Trager, W., and Jensen, J.B., 1976, *Science*, 193: 673-675.
19. Benoit, F., Valentin, A., Pellisier, Y., Marion C., and Dakuyo, Z., 1995, *Trans Roy. Soc. Trop. Med. Hyg.*, 89: 217-218.