IN VITRO ANTIPLASMODIAL ACTIVITY AND CYTOTOXICITY OF NEW N-BENZYL 1,10-PHENANTHROLINE DERIVATIVES

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ABSTRACT

Our preliminary study showed that 1,10-phenanthroline skeleton was active in vitro on chloroquine-resistant and sensitive strains of Plasmodium falciparum. Based on the skeleton, 3 derivatives of N-benzyl 1,10-phenanthroline: (1)-N-(4-methoxy-benzyl)-1,10-phenanthrolinium bromide (1), (1)-N-(4-ethoxy-benzyl)-1,10-phenanthrolinium chloride (2), and (1)-N-(4-ethoxy-benzyl)-1,10-phenanthrolinium bromide (3) have been synthesized. This study was conducted to evaluate the in vitro antiplasmodial activity and cytotoxicity of these compounds. The antiplasmodial activity was tested in vitro against two strains of P. falciparum, FCR-3 and D10 while their cytotoxicity was tested against Vero cell line. The parasite growth was estimated by counting the percentage of infected erythrocytes within a thin blood film prepared after 24 and 72 hours incubation with each compound tested under a light microscope. The Vero cell growth was estimated by counting the percentage of viable cell after 24 and 72 hours incubation with each compound tested use MTT assay. The control parasite or cell free from any compounds was referred as 100% growth. The IC50 value showing concentration inhibiting 50% at the parasite or cell growth was determined by probit analysis. The results showed that all 3 compounds have antiplasmodial activity with IC50 value 0.82-3.04, 0.77-2.75, and 0.73-1.31 µM respectively, and the cytotoxic/antiplasmodial ratio indicates that all 3 compounds have high selectivity, 41.99 -154.60 for compound (1), 101.44-196.60 for compound (2), and 81.37-199.98 for compound (3).

Keywords: 1,10-phenanthroline, P. falciparum, antiplasmodial, cytotoxicity

INTRODUCTION

Malaria is mosquito borne disease and is one of the major killer diseases of the world [1]. More than 100 million clinical cases of the disease occurred in the world and result at least in 1-2 million deaths annually [2]. Based on the data from Ministry of Health Republic of Indonesia in 2003, the annual parasite incidence in Java-Bali was 0.22 among 1000 population while the annual malaria incidence in outer Java-Bali was 21.8 among 1000 population. Moreover, chloroquine resistance has been widespread at some endemic areas in Indonesia with their percentage varied from 10-97% [3]. The increasing resistance of Plasmodium falciparum strains to currently available anti-malarial agents [4].

Halofantrine is an effective drug against chloroquine-resistant P. falciparum. However, the drug is incompletely and variably absorbed, being more bioavailable if taken with fatty food. Prolongation of QT wave in electrocardiograph is a risk factor for ventricular arrhythmias, which have been documented in patients taking halofantrine [5].

Based on the disadvantages of halofantrine, Yapi et al. [6] have synthesized diaza-analogs of phenanthrene derived from 3-amino, 5-amino, 6-amino, 8-aminoquinoline and 5-aminoisoquinoline and have tested their in vitro antiplasmodial activity. The results showed that among the tested molecules, 1,10-phenanthroline skeleton was the most active compound on the both chloroquine-resistant (FcB1) and sensitive (Nigerian) strains in vitro with IC50 about 0.13 µM. In this study, the antiplasmodial activity of 1,10-phenanthroline exhibited better activity after blocking the potential chelating site by N-alkylation. In our study, some compounds of N-alkyl and N-benzyl 1,10-phenanthroline derivatives showed antiplasmodial activity against P. falciparum [7] and P. berghei in mouse malaria model [8]. Based on these results, this study was conducted to evaluate the antiplasmodial activity and cytotoxicity of 3 new derivatives of N-benzyl-1,10-phenanthroline [9] i.e. (1)-N-(4-methoxy-benzyl)-1,10-phenanthrolinium bromide (1), (1)-N-(4-ethoxy-benzyl)-1,10-phenanthrolinium chloride (2), and (1)-N-(4-ethoxy-benzyl)-1,10-phenanthrolinium bromide (3).

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EXPERIMENTAL SECTION

Materials
Three derivatives of N-benzyl-1,10-phenanthroline: (1)-N-(4-methoxy-benzyl)-1,10-phenanthrolinium bromide (1), (1)-N-(4-ethoxy-benzyl)-1,10-phenanthrolinium chloride (2), and (1)-N-(4-ethoxy-benzyl)-1,10-phenanthrolinium bromide (3) have been synthesized by Supargiyono et al. [9]. The chemical structure of these compounds are shown in Figure 1.

Two strains of P. falciparum chloroquine-resistant, FCR-3 (IC50>100 nM) and chloroquine-sensitive, D10 strain (IC50<100 nM) were obtained from Eijkman Institute for Molecular Biology, Jakarta. The Vero cell line (kidney cells from the African green monkey) was obtained from Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta.

Procedure
Two strains of P. falciparum were cultured according to the modified method described by Trager and Jensen [10]. The parasites were maintained in vitro in human red blood cells (O2), diluted to 1-2% hematocrit in RPMI-1640 medium (Sigma-Aldrich Inc., USA), supplemented with (Sigma Chemical Co., USA) and 23.78 mM NaHCO3 (Sigma-Aldrich Inc., USA) and 7.68 mM HEPES supplemented with 10% human O2 serum. Parasite cultures were synchronized by 5% of D-Sorbitol (Sigma-Aldrich Inc., USA) in distilled water as reported by Lambros and Vanderberg [11]. The compounds were tested three times in triplicate of 96-well plates (Nunclon™, Germany) cultures at ring stage of 2% parasitaemia (with 3% hematocrit). The stock solution of the compound (1 mg/mL) was dissolved in 100 µL dimethyl sulfoxide (DMSO) (Merck, Germany) and then was diluted with 900 µL RPMI-1640 medium. For each testing, the parasite cultures were incubated with the compounds at various concentrations for 24 and 72 h. Parasite growth was estimated by counting parasitemia using thin blood smear and light microscope [12]. The control parasite free from any compounds was referred as 100% growth. Concentrations inhibiting 50% of the parasite (IC50) were determined by probit analysis using SPSS 10.0 for windows software.

Cytotoxicity of the compounds was assessed against Vero cell line. The Vero cell line was cultured in M199 medium (Gibco, Auckland) containing 10% fetal bovine serum (Sigma-Aldrich, USA). The cell was incubated in 5% of CO2 incubator, 37°C. Subcultures were obtained after treatment with 0.125% trypsin (Gibco, Auckland) in phosphate buffer saline. Vero cells were cultured in 96-well plates at 1 x 104 cells/well in 100 µL medium and incubated for 24 h. Then, 100 µL of compounds solution at various concentrations was added. Cell growth was estimated by MTT assay [13] to evaluate the cytotoxicity of the compounds after 24 h and 72 h incubation. The absorbance was measured at 595nm in the microplate reader (BIO-RAD). The cell numbers were calculated from absorbance using the standard curve. Concentrations inhibiting 50% of cell growth were determined by probit analysis using SPSS 10.0 for windows software.
Table 1. IC$_{50}$ (µM) of N-benzyl 1,10-phenanthrolines derivatives on FCR-3 and D10 of *P. falciparum* in vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>FCR-3</th>
<th>D10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
<td>24 h</td>
</tr>
<tr>
<td>1. (1)-N-(4-methoxy-benzyl)-1,10-phenanthroline bromide</td>
<td>0.82 ± 0.01</td>
<td>0.93 ± 0.02</td>
<td>3.04 ± 0.25</td>
</tr>
<tr>
<td>2. (1)-N-(4-ethoxy-benzyl)-1,10-phenanthroline chloride</td>
<td>1.38 ± 0.06</td>
<td>0.77 ± 0.02</td>
<td>2.75 ± 0.53</td>
</tr>
<tr>
<td>3. (1)-N-(4-ethoxy-benzyl)-1,10-phenanthroline bromide</td>
<td>1.12 ± 0.04</td>
<td>0.73 ± 0.03</td>
<td>1.11 ± 0.03</td>
</tr>
</tbody>
</table>

Table 2. IC$_{50}$ of N-benzyl 1,10-phenanthrolines derivatives on Vero Cell Line and Cytotoxic/antiplasmodial ratio on FCR-3 and D10 of *P. falciparum*

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM) on Vero Cell Line</th>
<th>CAR$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>(1)</td>
<td>127.26 ± 13.54</td>
<td>114.92 ± 2.74</td>
</tr>
<tr>
<td>(2)</td>
<td>270.96 ± 14.73</td>
<td>135.11 ± 7.07</td>
</tr>
<tr>
<td>(3)</td>
<td>223.19 ± 8.00</td>
<td>106.48 ± 0.67</td>
</tr>
</tbody>
</table>

$^a$Cytotoxic/antiplasmodial ratio on FCR-3 and D10 calculated for 24 h and 72 h of incubation time

RESULTS AND DISCUSSION

The 1,10-phenanthrolines ring system is well known for its metalloprotease inhibition activities by chelating divalent metal ions. As a chelating metal compound, 1,10-phenantroline has been used as antimicrobial agent against bacterial species such as *P. ruminicola*, *F. succinogenes*, *L. multipara* and *M. elsdenii* [14]. In addition, the antitumor activity of 1,10-phenanthroline was reported by Sakurai *et al.* [15]. A derivative of 1,10 phenanthroline, bis (4,7-dim ethyl-1,10-phenanthroline) sulfatooxovanadium (IV) exhibited apoptosis inducer activity in human cancer cells [16], antileukemic activity with matrix metalloproteinase inhibitor [17], and antitumor activity [18].

The anti-malarial activity of 1,10 phenanthroline was reported by Yapi *et al.* [6] and exhibited better activity after blocking the potential chelating site by N-alkylation. Some compounds of N-alkyl and N-benzyl 1,10-phenanthroline derivatives showed antiplasmodial activity against FCR-3 and D10 *P. falciparum* [7] and in mouse malaria model [8].

In this research, the chelating capacity of 1,10-phenantroline was blocked by N-1 benzylation. Two strains of *P. falciparum* chloroquine-resistant, FCR-3 (IC$_{50}$>100 nM) and chloroquine-sensitive, D10 strain (IC$_{50}$<100 nM) were used to evaluate the *in vitro* antiplasmodial activities of compound (1)-(3). The results are summarized in Table 1. The results showed that all 3 compounds have antiplasmodial activity with IC$_{50}$ value 0.82-3.04, 0.77-2.75, and 0.73-1.31 µM respectively. It was agree with Sholikhah *et al.* [7] and Wijayanti *et al.* [8] that the other derivatives of N-benzyl 1,10 phentantrone showed antiplasmodial activity. In this study, compound (1) more active (p<0.05) on FCR-3, chloroquine-resistant strain than on D10, chloroquine-sensitive strain of *P. falciparum*. However, compound (2) and (3) have the same activity (p>0.05) on both of strains.

*In vitro* cytotoxicity assay on Vero cell and calculation of the cytotoxic/antiplasmodial ratio (CAR) at 24 h and 72 h incubation time are summarized in Table 2. All the 3 compounds have high value of CAR i.e. 41.99-154.60 for compound (1), 101.44-196.60 for compound (2), and 81.37-199.98 for compound (3), indicating that these compounds have high selectivity.

CONCLUSION

The results showed that all 3 investigated compounds have antiplasmodial activity, and the cytotoxic/antiplasmodial ratio indicates that all 3 compounds have high selectivity. Further study will be conducted to evaluate *in vivo* antiplasmodial activity of all 3 investigated compounds on mice infected *P. berghei*.

ACKNOWLEDGEMENTS

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