



Research Article

SEMISYNTHESIS AND BIOLOGICAL EVALUATION OF EURYCOMANONE DERIVATIVES AS NEW ANTIMALARIAL

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Abstract: Several new eurycomanone derivatives were synthesized by esterification using isolated eurycomanone and acylating agent. Acylating agents which were used for esterification of isolated eurycomanone using acetylchloride, valerylchloride, butyrylchloride, methoxybenzoylchloride and succinicanhydride. Eurycomanone and its derivatives were used for in vitro antimalarial activity test. Testing of in vitro antimalarial activity was conducted by candle jar method. Eurycomanone and its derivatives in six levels concentration (0; 1; 10; 100; 1000; and 10.000 ng/mL) were incubated with *Plasmodium falciparum* strain 3D7 for 72 hours in CO₂ incubator at 37⁰ C. Antimalarial activity of eurycomanone and its derivatives were assessed microscopically after making thin blood smear and staining with Giemsa 10%. The fifty percent of *P. falciparum* growth inhibitory is determined by linear regression analysis. The result showed that eurycomanone more potent than chloroquine and its derivatives. Monoacyl eurycomanone more active than diacyl and triacyl eurycomanone.

Key words: Plasmodium falciparum, . Monoacyl eurycomanone, antimalarial activity

1. Introduction

World Health Organization has recommended research on natural product for treatment of diseases¹ included treatment of malaria. The ongoing spread of multidrug resistant strains of *Plasmodium falciparum* the most virulence malaria parasite, is a serious public health problem in the malaria endemic countries.² This situation has attracted attention the researchers in the world to find new antimalarial drugs. Several antimalarial drugs with various structures have been isolated from medicinal plants and play an important role in the development of new antimalarial drugs. Ethnopharmacological approaches can be the one of promising way to find new chemical compounds that could be used as templates for designing new drug derivatives with improved properties.³

Antimalarial drug discovery and development can follow several strategies, initially from structural modifications of existing drugs to the design of new novel drugs that act on new targets. The great challenge of drug discovery efforts are to identify, develop new compounds with high efficacy and safety, moreover to examine their mechanism of action. The chemical compounds of proven traditional medicine can also be used as starting materials for semisynthetic drugs. Generally, semisynthetic of proven antimalarial compound from traditional medicine is predicted to be a rapid strategy for discovery and development of new antimalarial drugs.³⁻⁶

This research article, discusses novel antimalarial drugs which is originated from Indonesian traditional medicine known as "Pasak bumi" (*Eurycoma longifolia*,

Jack.). The parts of this plant have been traditionally used for antimalarial, anticancer, antidiabetic, antipyretic, antimicrobial, and aphrodisiac activities.⁷⁻⁹

Several chemical compounds in pasak bumi roots have been identified and they are quassinoids, canthin-6-one alkaloids, β -carboline, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans.^{4,7} Previously, several antimalarial drugs in therapeutic use were isolated from traditional medicine and then modified their structures for preparing the new drugs with high potency, safety and other pharmacological properties.¹⁰

Eurycomanone is a chemical compound isolated from the extract of pasak bumi roots (*Eurycoma longifolia*, Jack.). Eurycomanone is a C₂₀ quassinoid with a molecular formula of C₂₀H₂₄O₉ and also used as bioactive marker for this plant.⁷⁻⁹ Previous studies about eurycomanone, showed that eurycomanone is the best potentiated in vitro antimalarial activity with IC₅₀ value 47,7 ng/mL on *P. falciparum* strain D6 and 48,1ng/ml on *P. falciparum* strain W2. Chan *et al* showed the eurycomanone has IC₅₀ 0,56 μ M on *P. falciparum* strain Gombak A and 0,10 μ M on *P. falciparum* strain D10.⁸ Due to eurycomanone have better antimalarial activity than chloroquine and its concentration in the roots higher than other compounds; Darise *et al* therefore it was selected for isolation and semisynthesized its derivatives.¹¹ Semisynthesized of eurycomanone derivatives were performed by replacement of hydroxyl groups with acylchloride and carboxylic anhydride groups, then the assessed of their *in vitro* antimalarial activity against *P. falciparum* strain 3D7 by candle jar method.¹²

2. Materials and Method

a. Isolation and semisynthesis of eurycomanone derivatives

The procedure for isolation of eurycomanone from pasak bumi roots has been described in previous publication.¹³ Semisynthesis of eurycomanone derivatives has been performed by simple esterification without using protecting agent. Several acylchloride and one of carboxylic anhydride were used for esterification isolated eurycomanone. Eurycomanone which is isolated from pasak bumi roots (50 mg, 0,1225 mmol) was dissolved in cold pyridine. The acylating agent (acetylchloride, butyrylchloride, valerylchloride, methoxybenzoyl chloride and succinic anhydride) in amount of 0,49 mmol were dissolved in cold chloroform respectively. The each acylating agent solution was added slowly to the eurycomanone solution at 0°C and the reaction mixture stirred for 1 hour in ice bath. The reaction mixture was refluxed and stirred using magnetic heat stirrer at 50°C – 60°C for 6-8 hour and every 2 hour checked the product by TLC. After acylating process ended, the mixture was added with 10 mL HCl 2 M and extracted three times with 10 ml of cold ethyl acetate. The ethyl acetate layer is washed three times with 10 mL cold water and then dried with sodium sulfate anhydrate. After filtering and drying the cooled precipitate is poured, crystallized in methanol and preparing for spectroscopic analysis.

b. Cultivation of *P. falciparum*

Plasmodium falciparum strain sensitive chloroquine 3D7 was maintained *in vitro* continuous culture.¹² Parasite culture was incubated at 37°C for 48 hours, and propagated in RPMI-1640 (Sigma-Aldrich) complete medium containing 3-5 % hemotocrit and 10% AB⁺ pooled human serum from uninfected-malaria volunteers. Human erythrocyte was also supplied from the same individual uninfected malaria volunteers. Erythrocyte was separated from its serum and washed in RPMI-1640 medium three times and used for 1 month. Pooled human serum from individual uninfected malaria, was incubated at 37°C for 4 hours and 1 hour at 0°C, then centrifuged to separate serum from erythrocyte. Serum was heated at 56°C for 1 hour to inactivate complement and stored at -20°C and warmed before used. Medium RPMI-1640 was supplemented with gentamicin (Sigma) 2.5 µg/ml, hypoxanthine (Sigma) 50 µg/ml, 25 mM HEPES (GibcoBRL) buffer and 25 mM Sodium bicarbonate (Merck) that will change the pH medium to approximately 7.4. Culture medium was prewarmed at 37°C before used and changed daily.

c. Testing of antimalarial activity

In vitro antimalarial activity test used “post-test only with control design”. RPMI 1640 used as culture medium for cultivation of *P. falciparum* strain 3D7. Treated drug were eurycomanone triacetate, eurycomanone dibutyrate, eurycomanone monovalerate, eurycomanone dimethoxybenzoate and eurycomanone disuccinate dissolved in dimethylsulfoxide 0,01%, added with medium and prepared into 6 levels concentration (0; 1; 10; 100; 1000; and 10.000 ng/mL). Negative control was culture medium, dimethylsulfoxide and the malarial parasites.

Positive control was chloroquine, culture medium and dimethylsulfoxide. The 96 well microplate was filled by 100 µL of *Plasmodium* in ring stadium with 0,5% parasitemia in medium complete with hematocrit 3%. Then 100 µL tested compounds with various concentration were added to microculture. The microplate was incubated for 72 hours in 5% CO₂ incubator at 37°C. After incubation, supernatant fluid was removed without disturbing the erythrocyte layer. Parasitemia was calculated by made the thin blood smear from the erythrocyte layer and stained with 10% Giemsa for 15 minutes. The *in vitro* antimalarial activity of the eurycomanone derivatives were calculated by counted the fifty percent of growth inhibition (IC₅₀) using linear regression analysis. *In vitro* antimalarial activity is expressed as the 50% inhibitory concentrations (IC₅₀), the drug concentration that produces 50% inhibition of *Plasmodium* growth *in vitro*. Each IC₅₀ value is expressed by the mean ± SD of at least three separate experiments performed in triplicate.

3. Result and Discussion

a. The result of isolation eurycomanone

In finding of new antimalarial compound was originated from pasak bumi roots (*E. longifolia*, Jack.), we macerated pasak bumi roots with methanol and after vacuum evaporating the liquid extract gave 4% solid extract. Fractionation were done to the extract by vacuum liquid chromatography (VLC) for obtaining a concentrated eurycomanone and yielded 1%. Isolation of eurycomanone from this fraction was performed by preparative thin layer chromatography (TLC-p) and yielded 0,02%.

b. The result of semisynthesized eurycomanone derivatives

The potentialized antimalarial eurycomanone was esterified by using acylchloride and carboxylic anhydride to influence their activity. Structural modification of eurycomanone by esterification is attempted with the aim of increasing activity, decreasing toxicity, or improving other pharmacological profiles. In finding new antimalarial drugs with better activity than previous compound, it was esterified OH group in eurycomanone structure by acetyl chloride, butyrylchloride, valerylchloride, methoxybenzoyl chloride and succinic anhydride. The result of esterification gave eurycomanone triacetate (65,25%), eurycomanone dibutyrate (60,35%), eurycomanone monovalerate (55,10%), eurycomanone dimethoxybenzoate (60,10%) and eurycomanone disuccinate (65,25%).

c. Identifying of eurycomanone and its derivatives by spectroscopic analysis

Chemical structure of all tested compounds had analyzed by spectroscopic analysis. Eurycomanone as starting material has formula C₂₀H₂₄O₉ (MW 408,02; m.p 254⁰-257⁰C) and its derivatives eurycomanone triacetate C₂₆H₃₀O₁₂ (MW 537,58; m.p 225-227⁰C), eurycomanone dibutyrate C₂₈H₃₆O₁₁ (MW 548,94; 241-243⁰C), eurycomanone monovalerate C₂₅H₃₂O₁₀ (MW 492,8; m.p 235-237⁰C), eurycomanone dimethoxybenzoate C₃₆H₃₆O₁₃ (MW 676,13; m.p 225-228⁰C) eurycomanone disuccinate C₂₈H₃₀O₁₅ (MW 606,86, m.p 251-254⁰C).

d. The result of in vitro antimalarial activity testing against *P. falciparum* strain 3D7

In vitro screening of antimalarial activity of eurycomanone and its derivatives is based on the ability of the compounds to inhibit the growth of *Plasmodium* in human erythrocytes culture. Testing of the compounds as antimalarial drug against chloroquine sensitive strain of *P. falciparum* 3D7 was conducted at 72 hours incubation period. The result of the test toward five semisynthesized compounds showed that eurycomanone has the best antimalarial activity. Monoacyl eurycomanone have higher activity than diacyl and triacyl eurycomanone. The IC_{50} value of eurycomanone and its derivatives is presented in Table 1.

Tabel 1. The IC_{50} value of eurycomanone and its derivatives against *P. falciparum* strain 3D7 after incubation 72 hours.

Senyawa uji	IC_{50} (μ M)
Eurikumanon	$4,7 \times 10^{-6}$
Eurikumanon triasetat	23130×10^{-6}
Eurikumanon dibutirat	7200×10^{-6}
Eurikumanon monovalerat	700×10^{-6}
Eurikumanon dimetoksibenzoat	8800×10^{-6}
Eurikumanon disuksinat	5500×10^{-6}
Klorokuin dipospat	00×10^{-6}

Eurycomanone and some of its derivatives have already shown as new potentiated antimalarial drug. Eurycomanone displays higher antimalarial activity than its derivatives. In the future, may be these drugs have an impact to solve the malarial problem especially to *P. falciparum* multi-resistant parasites. These results suggest some general structural requirements for optimal antimalarial activity of eurycomanone are influenced by: a) a five ring skeleton with a lactone D-ring; b) an α - β unsaturated ketone and an hydroxyl group next to the carbonyl in the A-ring; c) two free hydroxyl groups and a oxygen methylene bridge in the C-ring; and d) an ester group at either C-15 or C-12. The structural requirements for antimalarial activity is the structure in A-ring and C-15 ester group seems more important.¹⁴⁻¹⁷ The following criteria of in vitro antimalarial activity which is adopted from Batista *et al*¹⁸ for pure compound described that: the $IC_{50} < 1 \mu$ M, excellent/potent activity; IC_{50} of 1-20 μ M, good activity; IC_{50} of 20-100 μ M, moderate activity; IC_{50} of 100-200 μ M, low activity; and $IC_{50} > 200 \mu$ M, inactive.

Conclusion

The result showed that all eurycomanone derivatives have various antimalarial activity against *P. falciparum* strain 3D7: The IC_{50} values of eurycomanone $4,7 \times 10^{-6} \mu$ M; eurycomanone triacetate $23130 \times 10^{-6} \mu$ M; eurycomanone dibutyrate $7200 \times 10^{-6} \mu$ M; eurycomanone monovalerate $700 \times 10^{-6} \mu$ M; eurycomanone dimethoxybenzoate $8800 \times 10^{-6} \mu$ M; eurycomanone disuccinate $5500 \times 10^{-6} \mu$ M; and chloroquine diphosphate $10000 \times 10^{-6} \mu$ M.

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