In Vitro Antiplasmodial Activity of Brucein A Semisynthetic Compounds

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ABSTRACT

Introduction: Brucein A has been known to have antiplasmodial activity. Some new compounds were synthesized to increase their antiplasmodial activity, i.e 3-benzoyl brucein A, 3-dimethyl sulphate bruceine A, 3-choro benzoyl brucein A, and 3-chloro acetyl bruceine A. However, their antiplasmodial activity have not studied yet.
Objectives: To know the in vitro antiplasmodial activity of 3-benzoyl brucein A, 3-dimethyl sulphate bruceine A, 3-choro benzoyl brucein A, and 3-chloro acetyl bruceine A.
Methods: Antiplasmodial activity was conducted by incubating FCR-3 strain of P. falciparum with 3-benzoyl brucein A, 3-dimethyl sulphate bruceine A, 3-choro benzoyl brucein A, and 3-chloro acetyl bruceine A in various concentrations for 72 hours. Parasitemia after incubation period of each compound was calculated by making a thin smear stained with 5% Giemsa.
Results: Semisynthetic compounds of bruceine A have antiplasmodial activity in vitro with IC_{50} value were 2.648 ± 1.30 ng/mL for 3-benzoyl bruceine A, 1.098 ± 0.510 ng/mL for 3-dimethyl sulphate bruceine A, 50.246 ± 0.207 ng/mL for 3-chloro benzoyl bruceine A and 67.951 ± 11.517 ng/mL for 3-chloro acetyl brucein A. The IC_{50} value of Bruceine A as the lead compound was 3.87 ± 2.530 ng/mL
Conclusion: The 3-dimethyl sulphate bruceine A showed the highest antiplasmodial activity among 4 semisynthetic compounds of Bruceine A.

Keywords: bruceine A, semisynthetic compound, Plasmodium falciparum, in vitro antiplasmodial activity.

INTISARI

Pendahuluan: Brucein A dikenal memiliki aktivitas antiplasmodium yang dibuktikan pada uji antiplasmodium in vitro. Beberapa senyawa baru turunannya telah disintesis untuk meningkatkan aktivitasnya yaitu 3-benzoil brucein A, 3-dimetil sulfat bruceine A, 3-kloro benzoil brucein A dan 3-kloro asetil bruceine A, namun belum dibuktikan potensinya sebagai antiplasmodium.
Tujuan: Menguji aktivitas antiplasmodium in vitro senyawa 3-benzoil brucein A, 3-dimetil sulfate bruceine A, 3-kloro benzoil brucein A dan 3-kloro asetil bruceine A.
Metode: Uji aktivitas antiplasmodium in vitro dengan menggunakan P. falciparum FCR-3 untuk senyawa 3-benzoil brucein A, 3-dimetil sulfat bruceine A, 3-kloro benzoil brucein A dan 3-kloro asetil bruceine A pada berbagai peringkat konsentrasi dengan masa inkubasi 72 jam. Parastemia dihitung dari sediaan apus tipis yang di cat dengan Giemsa 5%.
Hasil: Aktivitas antiplasmodium in vitro senyawa semi sintetik brucein A didapatkan nilai IC_{50} sebesar 2,648 ± 1,30 ng/mL untuk 3-benzoil bruceine A, 1,098 ± 0510 ng/mL untuk 3-dimetil sulfat bruceine A, 50,246 ± 0,207 ng/mL untuk 3-kloro benzoil bruceine A dan 67,951 ± 11,517 ng/mL untuk 3-kloro asetil brucein A. Nilai IC_{50} Brucein A sebagai senyawa penuntun adalah 3,87 ± 2,530 ng/mL.

Simpulan: Senyawa 3-dimetil sulfat bruceine A mempunyai aktivitas antiplasmodium in vitro terbaik diantara keempat senyawa semi sintetik Brucein A

Kata kunci: bruceine A, senyawa semi sintetik, Plasmodium falciparum, aktivitas antiplasmodium in vitro.

INTRODUCTION

Malaria is one of serious infectious disease in the world. Drug resistance in malaria parasite is one of big obstacle on malaria eradication which cause therapeutic failure moreover death[1].

Chloroquine, one of well known antimalaria, with good therapeutic effect, low cost, low side effect and has been widely used was reported to be resistant now. That condition urges the development of new antimalaria agent. One strategy in developing new antimalaria agent is by synthesized new compound from lead compound which already known to have antiplasmodial activity. Lead compound are chosen based on chemical structure which has similarity with another compound or analogue compound that has already known having great antiplasmodial activity[2].

Bruceantin compound, brusatol; bruceine A; bruceine B; dan bruceine C are analogue compound of cuasinoid group which have antiplasmodial activity against *P. falciparum in vitro*. Bruceantin have lowest IC_{50} (0.0008 ìg/mL) consecutive with brusatol, bruceine C, bruceine A and bruceine B. All of those IC_{50} were lower than chloroquine 0.210 ìg/mL[3,4]. Bruceantin compound also have antiplasmodial activity *in vivo* against *Plasmodium berghei*[3].

Another research showed that bruceantine and brusatol as bruceine A analogue has been modified its chemical structure for antimalaria, antimicrobia, and also anticancer[5]. Based on research, bruceine A isolated from *Brucea javanica*, L., Merr. as a lead compound in antimalaria development. Chemical structure modification of bruceine A was conducted by substitute benzyl chloride pharmacophor, dimethyl sulphate, chloro benzoic chloride, and chloro acetyl chloride on its structure and become semi synthetic compound as consecutively 3-benzoic bruceine A, 3-dimethyl sulphate bruceine A, 3-chloro benzoic bruceine A, and 3- chloro acetyl bruceine A[6]. The substituent addition was due to enhance its interaction to *Plasmodium* with high specificity and low toxicity against the host[7]. All the modified compound of bruceine A (3-benzoic bruceine A, 3-dimethyl sulphate bruceine A, 3-chloro benzoic bruceine A, and 3- chloro acetyl bruceine A) never tested on its antiplasmodial activity assay.

MATERIALS AND METHODS

Antiplasmodial activity *in vitro* assay of bruceine A and its semisyntetic compound

a. Plasmodium falciparum culture

P. falciparum culture was performed using Trager & Jensen method[9,10]. Malaria culture medium (MCM) was composed of 10.43g RPMI 1640 powder, 6g HEPES, 2g NaHCO3, 25mg gentamycin in 1 liter of sterilized aquadest. Medium was sterilized using micro filter 0.22ìm pH ± 7.2 and stored at 4^0C. O blood erythrocytes were used as parasite medium to grow. Erythrocytes were washed 3 times to eliminate anticoagulant, serum, and leucocytes. Packed erythrocytes were then stored at 4 °C for 14 days.

P. falciparum strain FCR-3 was thawed from liquid nitrogen storage by adding NaCl 12% after 3 minute warmth. Ten mL of NaCl 1.6% was added for every 1mL *Plasmodium* suspension followed by centrifugation to separate the supernatant. Ten mL of 0.2% dextrose in 0.9% NaCl was added followed by centrifugation to get the *Plasmodium* pellet.

Complete MCM (cMCM) was made by adding 10 mL malaria culture medium into culture flask, mix with 10% O blood human serum. Thawed *Plasmodium* was poured into a culture flask that

containing cMCM and 50 ìL of normal erythrocyte. Culture flask were kept in the candle jar. As nitrogen source, candle was lit and the jar was then closed when the candle off. Candle jar was kept inside CO_2 incubator (37^0C) and medium was replaced at every 24 hours.

b. Antiplasmodial activity assay

For this assay, synchronized ring stadium was used[10]. Plasmodium with 2% parasitemia, 3% haematocrit was used for brucein A *in vitro* assay. Bruceine A compound; 3-benzoil bruceine A, 3-dimethyl sulphate bruceine A, 3-chloro benzoyl bruceine A, and 3-chloro acetyl bruceine A were dissolved in DMSO solution and diluted with RPMI. Samples concentration were 1, 2, 4, 8, 16 ng/mL for bruceine A, 3-benzoil bruceine A, 3-dimethyl sulphate bruceine A. For 3- chloro acetyl bruceine A, the sample concentration were 20, 40, 80, 160, 320 ng/mL. RPMI was used for negative control. Chloroquin was used to explore its sensitivity against *P. falciparum* with concentration 4, 8, 12,16 and 20 Kg/mL. Sample solutions (100µL) with various concentration were put into 96 well micro plate with added 100µL of infected erythrocytes. Each concentration was replicated 3 times. Each sample was tested duplex except for 3-chloro acetyl bruceine A (once). Micro plate was kept into candle jar and incubated at 37°C for 72 hours. At the end of incubation time, thin smear of *P. falciparum* was made for each well, 5% Giemsa stained and examined under microscope. Parasitaemia and percentage of parasite growth inhibition were calculated for each well.

c. Statistical analysis

Probit analysis was utilized to determine antiplasmodial activity which expressed as IC_{50}.

RESULTS AND DISCUSSIONS

Antiplasmodial activity *in vitro* assay of brucein A and its derivates

Bruceine A and its derivates 3-benzoil bruceine A, 3-dimethyl sulphate bruceine A, 3-chloro benzoyl bruceine A, and 3-chloro acetyl bruceine A were tested its antiplasmodial activity *in vitro* against chloroquine-sensitive *P. falciparum*. *P. falciparum* growth was evaluated using thin smear and examined under microscope. Chloroquine was used a positive control and DMSO was used a solvent control. Results of antiplasmodial activity of some compounds and chloroquine were listed in Table 1.

Antiplasmodial activity was expressed as IC_{50}. The IC_{50} for 3-benzoil bruceine A was 2.648 ± 1.30 ng/mL; 3-dimethyl sulphate bruceine A was 1.098 ± 0.510 ng/mL; 3-chloro benzoyl bruceine A was 50.246 ± 0.207 ng/mL; 3-chloro acetyl bruceine A was 67.951 ± 11.517 ng/mL; and bruceine A as lead compound was 3.87 ± 2.530 ng/mL. The IC_{50} of chloroquine as positive control was 4.808 ± 0.096 ng/mL.

Antiplasmodial activity *in vitro* assay is an early exploration stage for compound which will develop as antimalaria. Bruceine A and its derivates was examined its antiplasmodial activity against *P. falciparum* chloroquine sensitive. Bruceine A was a lead compound that possessed antiplasmodial activity and also its derivates. Bruceine A was modified by substitution of pharmacophor group (benzoic chloride, dimethyl sulphate, chloro benzoic chloride, and chloro acetyl chloride) into its derivates 3-benzoil bruceine A, 3-dimethyl sulphate bruceine A, 3-chloro benzoyl bruceine A, and 3- chloro acetyl bruceine A. Those new compounds potential for further antimalarial drug development. Bruceine A, 3-benzoil bruceine A, and 3-dimethyl sulphate bruceine A possessed lower IC_{50} level than chloroquine and both derivates also possessed lower IC_{50} level than the lead compound, bruceine A.

Based on Egan[10] requirements, a compound stated having poor activity or has no effect if the IC_{50} level 20 times higher than chloroquine's IC_{50}[10]. The IC50 of chloroquine was 4.08 ± 0.096 ng/mL and twenty times of it was 96.16 ng/mL. The IC_{50} of bruceine A and all the derivates were lower than chloroquine based on Egan criteria[10]. Hence those compounds are potential to be further investigated.

Table 1. Antiplasmodial activity of Bruceine A and it derivates against *P. falciparum* FCR-3 strain in *in vitro* assay

Compounds	Concentration (ng/mL)	% Parasitaemia	% growth inhibition ± SD
Bruceine A	1	5.343 ± 5.410	53.967 ± 45.474
	2	3.715 ± 2.803	67.859 ± 22.924
	4	2.226 ± 0.708	80.279 ± 5.324
	8	1.533 ± 0.283	86.373 ± 3.246
	16	1.566 ± 0.509	86.102 ± 5.100
3-benzoyl bruceine A	1	10.098 ± 3.213	0.34 ± 22.022
	2	8.175 ± 3.036	19.319 ± 21.926
	4	7.842 ± 3.971	23.295 ± 31.725
	8	6.051 ± 5.339	42.015 ± 47.593
	16	5.398 ± 6.536	49.549 ± 29.716
3-dimethyl sulphate bruceine A	1	6.824 ± 1.696	40.477 ± 5.026
	2	4.020 ± 1,558	65.044 ± 9.575
	4	1.558 ± 0,424	86.574 ± 1.559
	8	1.364 ± 0,605	86.630 ± 5.238
	16	1.552 ± 0,493	85.987 ± 6.348
3-chloro acetyl bruceine A	10	11. 499 ± 1,853	33.624 ±10.697
	50	8.782 ± 0.649	49.312 ± 3.893
	100	8.524 ± 1.607	50.799 ± 9.279
	500	6.836 ± 0.927	60.540 ± 5.352
	1000	4.205 ± 1.838	75.728 ± 10.607
3-chloro benzoyl bruceine A	20	9.137 ± 3.653	5.08 ± 3.961
	40	7.717 ± 4.393	22.001 ± 17.646
	80	5.737 ± 5.183	45.958 ± 35.083
	160	3.071 ± 3.952	73.915 ± 31.958
	320	0.591 ± 0.634	94.590 ± 4.835
Chloroquine	4	5.963 ± 0,532	26.609 ± 6.556
	8	0.303 ± 0,343	96.262 ± 4.223
	12	0.204 ± 0,285	97.481 ± 3.512
	16	0.161 ± 0,278	98.018 ± 3.431
	20	0.444 ± 0,076	99.454 ± 0.945
	40	0.000 ± 0,000	100 ± 0.000

CONCLUSION

This research showed that all of Bruceine A derivates have potential antiplasmodial activity *in vitro*. Further study is needed for *in vitro* cytotoxicity of bruceine A semisynthetic compound on normal cells.

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REFERENCES

1. World Health Organization. *World Malaria Report*. World Health Organization, Geneva. 2008.
2. Rosenthal PJ. Review: Antimalarial drug discovery: old and new approaches, *J.Exp.Biol*, 2003;206:3735-44.
3. O'Neill MJ, Bray DH, Boardman P, Chan KL, Phillipson JD, Warhurst DC, Peters W. Plants as Sources of Antimalarial Drugs, Part 4: Activity of Brucea javanica fruits against chloroquine-resistant *Plasmodium falciparum in vitro* and against *Plasmodium berghei in vivo*. *J. Nat. Prod*, 1987;50(1):41-8.
4. Roberts MF, Brucea spp.: *In vitro* culture and the production of canthinone alkaloids and other secondary metabolites. In: Y.P.S.Bajaj (Ed.): *Medical and Aromatic Plants VI*, pp: 23,31. Springer-Verlag, New York, 1994.
5. Guo Z, Vangapandu S, Sindelar RW, Walker LA, Sindelar RD. Biologically active quassinoids and their chemistry: Potential leads for drug design. *J. Med. Chem*, 2009;4:285-308.
6. Mustofa, Wijayanti MA, Tahir I, Mangunsong S. Aktivitas antiplasmodial, toksisitas *in vitro* dan analisis hubungan kuantitatif struktur aktivitas (HKSA) senyawa semisintetik golongan kuasinoid hasil isolasi dari buah Makasar (*Brucea javanica*, L.,Merr). *Research report*. Faculty of Medicine, Universitas Gadjah Mada, 2009.
7. Lang PT, Aynechi T, Moustakas D, Shoichet B, Kuntz ID, Brooijmans N, Oshiro CM. Molecular Docking and Structure-Based Design in: Huang, Z., (Ed.): *Drug Discovery Research* - New Frontiers in The Post-Genomic Era. pp: 3-4. Willey Interscience A Jhon Wiley & Sons, Inc., Publication, LEA & Febiger. San Diego, California, 2007.
8. Trager W, Jensen JB. Human malaria parasites in continuos culture. *Science,* 1976 ;193:673-5.
9. Ljungtröm I, Perlmann H, Schichtherle M, Scherf A, Wahlgren M. *Methods in Malaria Research*. 4th ed. Manassas: MR4/ATCC, 2004.
10. Egan TJ. Structure-Function Relationships in Chloroquine and Related 4-Aminoquinoline Anti-malarials, *Mini Reviews in Med.* Chem, 2001;1:113-23