

Synthesis of 2- and 3-methoxy-substituted Amino Chalcone Derivatives and Evaluation of Their Antimalarial Activity

Rahma Dona¹, Ihsan Ikhtiarudin¹, Melzi Octaviani¹, Enda Mora¹, Putri Johanda¹, Laras Maharani¹, and Cindy Fatma Yunita^{1*}

¹Sekolah Tinggi Ilmu Farmasi Riau, Jalan Kamboja, Kelurahan Simpang Baru, Pekanbaru, Indonesia

Corresponding Author:

Cindy Fatma Yunita

cindyfatma@stifar-riau.ac.id

Received: Apr 2026

Accepted: Sep 2026

Published: Sep 2026

© Cindy Fatma Yunita et al.
This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

This study involved the synthesis of chalcone analogues with variations in benzaldehyde (A; B; C), the characterisation of the compounds, and in vitro testing of their antimalarial activity. The aim of this study was to investigate the antimalarial potential of dimethoxy and trimethoxy amino group variations in chalcone derivatives. The synthesis was carried out using Claisen-Schmidt condensation reaction with stirring method for 7 hours at room temperature, using NaOH 40%(b/v) base catalyst and ethanol solvent p.a. The synthesised compound was purified, obtained orange yellow pure compound with yield of 19,42-50,19%. Purity tests using TLC, melting point and HPLC showed high purity. Structural characterization using UV-Vis, FT-IR, and ¹H-NMR spectroscopy verified the successful formation of the targeted chalcone analogue structures. Antimalarial activity test showed the compounds A, B, and C inhibited the polymerisation of hemin to β -hematin with IC₅₀ values of 107,30 μ g/mL (378,71 μ M); 79,89 μ g/mL (282 μ M); 57,47 μ g/mL (183,41 μ M), respectively better than the positive control hydroxychloroquine sulfate with IC₅₀ value of 187,02 ppm (430,9 μ M). From these results, differences in the position of the methoxy group in chalcone derivatives also result in differences in antimalarial activity, with compound C showing better inhibitory potential, followed by B and A respectively.

Keywords: Amino Methoxy Chalcone; Synthesis; Antimalarial; In Vitro; Hemin Polymerization.

Introduction

Malaria is an infectious disease that mostly occurs in tropical countries. Malaria is caused by parasites of the genus *Plasmodium* and is transmitted to humans through the bite of an infected female *Anopheles* mosquito. The disease is preventable and treatable. However, if not diagnosed quickly and treated properly, a mild case of malaria can turn into a severe illness and if left untreated can lead to death^[1]. The World Malaria Report 2023 recorded approximately 249 million malaria cases and 608,000 deaths worldwide in 2022^[1].

The pathogenesis of malaria is closely linked to the parasite's life cycle inside erythrocytes. In its food vacuole, *Plasmodium* degrades hemoglobin to obtain amino acids and releases free heme as a by-product. Free heme is toxic because it causes oxidative damage to the membranes and organelles of the parasite. To protect itself, the parasite detoxifies heme into hemozoin, an insoluble and non-toxic crystalline malaria pigment. Inhibition of hemozoin formation causes free heme to accumulate and kills the parasite^[3]. This detoxification pathway is absent in the human host, which makes it a selective target for antimalarial drugs. In laboratory studies, β -hematin, the synthetic form of hemozoin, is used

as a model to test inhibitors of hemozoin formation. Several studies have shown that compounds capable of inhibiting β -hematin formation are potential antimalarial drug candidates^[3].

Commonly used malaria treatments rely on blood schizonticides, which prevent and terminate clinical attacks, such as chloroquine, quinine, sulfonamides, tetracycline, atovaquone, and artemisinin and its derivatives^[4]. However, resistance has developed against several antimalarial drugs^[5]. Chloroquine resistant *Plasmodium falciparum* was first discovered in 1973 in East Kalimantan and *Plasmodium vivax* was first discovered in Nias (Papua) in 1991^[6]. Resistance to chloroquine and artemisinin can worsen the epidemiological situation and hinder malaria control^[7]. Therefore, new antimalarial compounds that can overcome resistance are urgently needed. Over the past 15 years, efforts have included vaccine development, clinical trials, drug identification, dose finding, and evaluation of single and combination therapies^[8]. One approach to antimalarial drug development is to explore natural materials and synthesize new compounds to reduce side effects^[9]. Inhibition of heme polymerization is regarded as a promising mechanism for designing drugs that can overcome multidrug resistance^[10].

One group of compounds that has been widely explored is the chalcones, secondary metabolites used to synthesize new and more effective drugs^[11]. Chalcone (IUPAC name 1,3-diphenyl-2-propen-1-one) is a precursor of flavonoids and isoflavonoids found in several medicinal plants^[12]. Chalcones and their derivatives show various pharmacological activities, including anti-inflammatory^[13], antiproliferative^[14], antimicrobial^[15], antidiabetic^[16], and antimalarial^[17] activities. Chalcones consist of two aryl groups linked by an α,β -unsaturated ketone, a simple structure that is easy to synthesize and modify for drug development^[12]. Theoretically, prenyl, allyl, alkoxy, and hydroxy groups increase lipophilicity, which is important for antimalarial activity. Hydroxy, alkoxy, and allyloxy groups have also been shown to enhance the

antimalarial activity of chalcone derivatives against *P. falciparum* 3D7^[18].

Amino- and methoxy- substituted chalcones are of particular interest because the amino group can form electrostatic interactions with residues of *P. falciparum* proteins. Suwito et al. reported that the amino group in methoxy chalcone derivatives plays an important role in inhibiting the interaction between ferredoxin and ferredoxin-NADP⁺ reductase, with the highest inhibition (50%) shown by (*E*)-1-(4-aminophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one^[19]. Setyarini and Astuti reported that (*E*)-1-(4-aminophenyl)-3-(2,3dimethoxyphenyl)prop-2-en-1-one was active against *P. falciparum* FCR-3 (IC₅₀ 14.86 $\pm$ 6.58 μ M)^[20]. Dona et al. reported that chalcones with an amino group on ring A and methoxy groups on ring B inhibited heme polymerization, with the best IC₅₀ of 115.18 μ g/mL for the 3,4-dimethoxy derivative^[21]. Fajrin reported that a chalcone derivative bearing 2,4-dimethoxy groups was active as an antimalarial (IC₅₀ 4.05 μ M)^[22].

Nevertheless, the activity of amino-methoxy chalcones has mostly been evaluated against parasites or specific protein targets, whereas data on heme polymerization inhibition remain limited. The effect of the position and number of methoxy groups on ring B on heme polymerization inhibition has not been studied systematically, because existing studies cover only a few substitution patterns, such as 3,4-dimethoxy. To the best of our knowledge, based on a literature search, the synthesis of 4'-amino-2,3-dimethoxychalcone, 4'-amino-2,4-dimethoxychalcone, and 4'-amino-2,4,5-trimethoxychalcone, together with their inhibitory activity against heme polymerization, has not been reported.

This study aimed to synthesize 4'-amino-2,3-dimethoxychalcone (A), 4'-amino-2,4-dimethoxychalcone (B), and 4'-amino-2,4,5-trimethoxychalcone (C) from 4'-aminoacetophenone and the corresponding benzaldehyde derivatives through Claisen-Schmidt condensation using 40% (w/v) NaOH as a catalyst and the stirring method; to confirm the structures of the synthesized compounds using UV-Vis, FT-IR, and ¹H-NMR spectroscopy; and to evaluate the *in vitro*

antimalarial activity of compounds A, B, and C through inhibition of hemin polymerization to β -hemin, based on IC_{50} values compared with the positive control hydroxychloroquine sulfate.

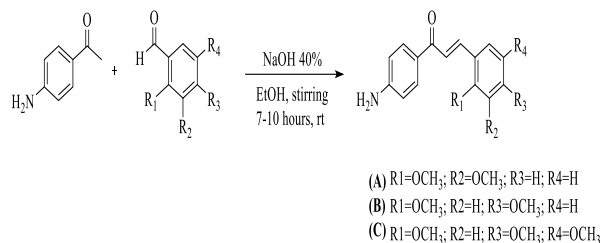


Figure 1. Synthesis route for compounds A, B, and C.

Experimental

Equipments

The equipment used in this study consisted of an analytical balance (Shimadzu®), hotplate magnetic stirrer (Thermo Cimatec™), universal indicators (Merck®), silica gel 60 F254 TLC plates (Merck®), UV cabinets at 254 and 366 nm (Camag®), Digital Melting Point-800 (DMP-800), High Performance Liquid Chromatography (HPLC) (Shimadzu® LC20 AT), UV-Vis spectrophotometer (Shimadzu®1900i), FT-IR spectrophotometer (Shimadzu® IR Spirit-A224158), ¹H-NMR spectrometer (Agilent 500 MHz), microplate reader (Epoch BioTek®), incubator (Memmert®) and laboratory glassware for synthesis and other antimalarial test.

Materials

The materials used in this study were 4'-aminoacetophenone (Sigma-Aldrich®), 2,3-dimethoxybenzaldehyde (Sigma-Aldrich®), 2,4-dimethoxybenzaldehyde (Sigma-Aldrich®), 2,4,5-trimethoxybenzaldehyde (Sigma Aldrich®), NaOH (Merck®), Tween-20 (Sigma-Aldrich®), acetate buffer, hemin (ferri protoporphyrin IX chloride), hydroxychloroquine sulphate (Hyloquin®), HCl (Merck®), acetonitrile for HPLC (Merck®), ethanol *p.a* (Merck®), DMSO (Sigma-Aldrich®), waterone (onemed®), and distilled organic solvents such as *n*-hexane, ethyl acetate and methanol.

Methods

Synthesis Of Amino Methoxy Chalcone Derivatives (A, B, and C)

Three amino methoxy chalcone analogue compounds (A, B, and C) derivatives were designed as depicted in **Figure 1**, synthesis using Claisen-Schmidt condensation method with NaOH base catalyst. The synthesis started with 10 mmol of 4'-aminoacetophenone and 10 mmol of methoxy benzaldehyde (2,3-dimethoxy; 2,4-dimethoxy; 2,4,5-trimethoxy) dissolved in ethanol *p.a* (60 mL) and stirred for 30 minutes. NaOH 40% (20 mL) was added slowly and then stirred at room temperature (15-30°C) for 7-10 hours. Monitor the progress of the reaction with TLC every hour. After the reaction was complete, the reaction mixture was added with cold aqua DM (30 mL) then neutralised with sufficient cold 6 N HCl to pH 5-7 (neutral), then cooled in an ice bath until a precipitate formed. The precipitate was filtered, then washed with cold Aqua DM and cold *n*-hexane. The crude product solids obtained were dried and checked for purity.

The purification of compound A was carried out by recrystallisation from hot ethyl acetate *p.a* (40-60°C). By dissolving the crude product and then dissolved with hot ethyl acetate *p.a* until a saturated solution is formed. The solution was filtered and dried. For purification of compounds B and C using column chromatography with eluent *n*-hexane, ethyl acetate and methanol as mobile phase with the help of nitrogen gas. The separation results were collected in vials, separated according to the colour bands formed, and then monitored using TLC throughout the elution process, with the R_f value serving as a reference for identifying the fractions containing the target compounds. The pure compounds derived from amino methoxy chalcone (A, B and C) obtained orange yellow, then weighed and the percentage yield calculated, yielding a yield of 19,42-50,19%. The purity and characterizations of compounds were carried out through using TLC, HPLC, melting point test, UV-Vis, FT-IR and ¹H-NMR.

In Vitro Antimalarial Activity Test

In vitro testing of antimalarial activity was conducted by inhibiting the polymerisation of hemin into β -hematin by following the modified procedure from^[3]. 90 μ L of compound solutions with varying concentrations or positive control solution of hydroxychloroquine sulphate was added to the wells, followed by 90 μ L of Tween-20 to each well. Then, 90 μ L of hemin solution dissolved in 1 mL of DMSO and diluted with acetate buffer (pH 4,8) was added. The mixture was then incubated at 37°C for 250 minutes. Absorbance measurements were taken at wavelengths of 415 nm and 630 nm.

Data Analysis

The antimalarial effectiveness is indicated by IC_{50} values derived from the linear regression equation $y = ax \pm b$, which is determined by calculating the fraction of heme that converted to β -hematin using the following formula:

$$f = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}} - A_{\text{min}}}$$

Where A_{control} represented heme absorption in the absence of Tween-20 or the sample, A_{sample} measures heme absorption when Tween-20 and sample are added, and A_{min} represented heme absorption with Tween-20 without sample.

The percentage of antimalarial inhibition activity is calculated using the formula:

$$\% \text{ inhibition} = (1-f) \times 100\%$$

Results and Discussion

Synthesis of Compounds A, B, and C

The A, B, and C chalcone derivatives have been successfully synthesised and characterised. The synthesis of A ((*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one, molecular formula $C_{17}H_{17}NO_3$) yielded an orange-yellow solid with a yield of 50,19%. Melting point 141,8–142,8°C. TLC analysis: $R_f = 0,5$ (*n*-hexane:ethyl acetate = 6:4). HPLC analysis showed a single peak at $t_R = 6,002$ minutes (isocratic elution, detector 364 nm). The UV spectrum (in *etOH*) was obtained at $\lambda_{\text{max}} = 364$ nm (absorbance = 0,872) and 251 nm (absorbance = 0,402). FT-IR: 3463–

3228, 2834, 1629, 1598. $^1\text{H-NMR}$ (Agilent 500 MHz, DMSO-d_6) showed ten signals corresponding to seventeen hydrogen atoms. These signals were observed at chemical shifts/ δ (ppm): 3,83 (s, 3H, $-\text{OCH}_3$); 3,77 (s, 3H, $-\text{OCH}_3$); 7,57 (d, 1H, $J = 7,15$ Hz); 7,11 (d, 1H, $J = 8,1$ Hz); 7,14 (d, 1H, $J = 8,1$ Hz); 7,82 (d, 1H, $J = 15,8$ Hz); 7,87 (d, 1H, $J = 15,7$ Hz); 7,91 (d, 2H, $J = 8,6$ Hz); 6,62 (d, 2H, $J = 6,85$ Hz); and 6,17 (s, 2H, $-\text{NH}_2$).

Compound B ((*E*)-1-(4-aminophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one molecular formula $C_{17}H_{17}NO_3$), obtained as an orange-yellow solid with a yield of 31,18%. Melting point 146,7–147,7°C. TLC analysis: $R_f = 0,375$ (*n*-hexane:ethyl acetate = 6:4). HPLC analysis showed a single peak at $t_R = 6,245$ minutes (isocratic elution, detector 370 nm). The UV spectrum (in *etOH*) was obtained at $\lambda_{\text{max}} = 380$ nm (absorbance = 0,766) and 250 nm (absorbance = 0,338). FT-IR: 3421–3347, 2836, 1633, 1594. $^1\text{H-NMR}$ (Agilent 500 MHz, DMSO-d_6) showed ten signals corresponding to seventeen hydrogen atoms. These signals were observed at chemical shifts/ δ (ppm): 6,61 (m, 1H); 6,59 (d, 1H, $J = 2,5$ Hz); 7,89 (s, 1H, $J = 6,5$ Hz); 3,89 (s, 3H); 3,82 (s, 3H); 7,69 (d, 1H, $J = 15,5$ Hz); 7,89 (d, 1H, $J = 15,5$ Hz); 7,88 (d, 2H, $J = 9$ Hz); 6,62 (m, 2H, $J = 10$ Hz); and 6,10 (s, 2H).

Compound C ((*E*)-1-(4-aminophenyl)-3-(2,4,5-dimethoxyphenyl)prop-2-en-1-one molecular formula $C_{18}H_{19}NO_4$), obtained as an orange solid with a yield of 19,422%. Melting point 201,7–202,7°C. TLC analysis: $R_f = 0,325$ (*n*-hexane:ethyl acetate = 6:4). HPLC analysis showed a single peak at $t_R = 4,937$ minutes (isocratic elution, detector 370 nm). The UV spectrum (in *etOH*) was obtained at $\lambda_{\text{max}} = 392$ nm (absorbance = 0,634) and 247 nm (absorbance = 0,275). FT-IR: 3423–3347, 2830, 1633, 1594. $^1\text{H-NMR}$ (Agilent 500 MHz, DMSO-d_6) showed ten signals corresponding to eighteen hydrogen atoms. These signals were found at chemical shifts/ δ (ppm): 6,61 (m, 1H); 3,85 (s, 3H, $-\text{OCH}_3$); 3,81 (s, 3H, $-\text{OCH}_3$); 3,88 (s, 3H, $-\text{OCH}_3$); 6,72 (s, 1H); 7,47 (s, 1H); 7,69 (d, 1H, $J = 15,6$ Hz); 7,93 (m, 1H, $J = 15,65$ Hz); 7,91 (d, 2H); 6,61 (d, 2H, $J = 8,65$ Hz); and 6,08 (s, 2H, $-\text{NH}_2$).

Amino chalcone derivatives (A, B, and C) were synthesised using a 40% NaOH base catalyst with a stirring method. The synthesis reactions of the three compounds are illustrated in **Figure 1**. The synthesis of compounds A, B, and C was carried out by reacting 4'-aminoacetophenone with benzaldehyde derivatives containing methoxy substituents at various positions at room temperature. The synthesis yielded solid compounds with high yields ranging from 72% to 89%. The crude product is the solid obtained immediately after neutralisation, filtration and washing. This solid is still a mixture containing the target chalcone alongside residual starting materials, reaction by-products and salt residues; consequently, its yield does not fully reflect the amount of the target compound.

Table 1. Interpretation of FT-IR spectroscopic data of compounds A, B, and C.

Comp	Wavenumber (cm ⁻¹)			
	NH ₂	C-H meth- oxy	C=O ke- tone	C=C aro- matic
A	2464- 3228	2834	1629	1598
B	3421- 3347	2836	1633	1594
C	3423- 3347	2830	1633	1594

Therefore, the crude product has not yet been characterised and is not used for activity testing. The crude products were purified by recrystallisation (A) using ethyl acetate as the solvent and

column chromatography (B and C) using nitrogen gas with *n*-hexane, ethyl acetate, and methanol as solvents. The purification process of compounds A, B, and C produced pure compounds with yields ranging from 19% to 50%, lower than the crude yield. The difference between the crude yield and the pure isolate is not entirely due to loss of the target compound, as part of the crude mass consists of non-target components which are indeed removed during the purification stage. The yield of the pure isolate decreased from A (50,19%) to B (31,18%) and C (19,42%). This trend is likely influenced by the electronic and steric effects of the methoxy groups on benzaldehyde. The methoxy groups at the ortho and para positions act as electron donors via resonance, thereby reducing the electrophilicity of the carbonyl carbon, whilst the ortho substituent imposes steric hindrance to enolate attack. Compounds B and C both possess methoxy groups in the ortho and para positions, whilst A only has an ortho group and a meta group, which act as inductive electron-withdrawing groups. This is consistent with A having the highest yield, as the lower conversion rates for B and C result in more starting material remaining that must be separated.

The low product yield obtained at this stage is generally due to several factors, such as the formation of certain by-products during the synthesis of compound B. Furthermore, a mismatch between the polarity of the solvent and that of the target compound can prevent the compound from being fully eluted. Consequently, the low

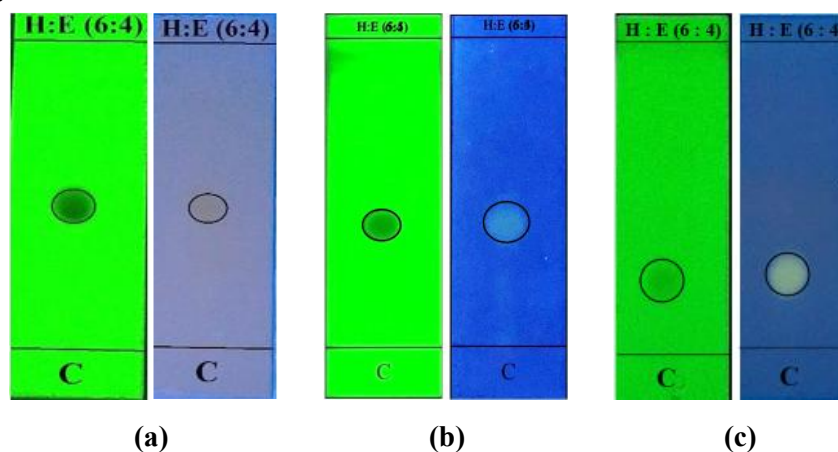


Figure 2. Chromatogram results of TLC tests of compounds; (a) A; (b) B; and (c) C

yield is primarily due to interactions between the compound and the stationary phase, the instability of the compound, and technical errors during the purification process using column chromatography^[23].

The purity of the compounds was determined through TLC testing, HPLC analysis and melting point measurement. The TLC test results of the compounds after purification can be seen in **Figure 2**. On the TLC plate eluted using *n*-hexane:ethyl acetate (6:4) mobile phase, a single spot was obtained, where R_f A (0.5) > B (0.375) > C (0.325). This sequence indicates that polarity increases from A to C, in line with the increasing number of oxygen atoms capable of forming hydrogen bonds with silica gel. The HPLC chromatogram shows a single peak for all three compounds in **Figure 3**. The retention time for C

Vis, FT-IR and ¹H-NMR analyses. The spectrum of chalcone compounds exhibits two absorption bands: band I (cinnamoyl conjugation) in the 330–550 nm range and band II (benzoyl conjugation) in the 250–280 nm range^[26]. Band I of compounds A, B and C appears at 364, 380 and 392 nm, whilst Band II remains virtually unchanged (251, 250 and 247 nm). Band II is relatively constant because the benzoyl system (ring A and the carbonyl group) is the same in all three compounds, whilst the bathochromic shift of Band I reflects differences in the substitution on ring B. The methoxy groups in the ortho and para positions relative to the vinyl chain (compounds B and C) enhance the delocalisation of π electrons via resonance, thereby reducing the $\pi \rightarrow \pi^*$ transition energy gap, whilst compound A possesses only ortho and meta groups, resulting in the shortest maximum absorption. The greatest shift

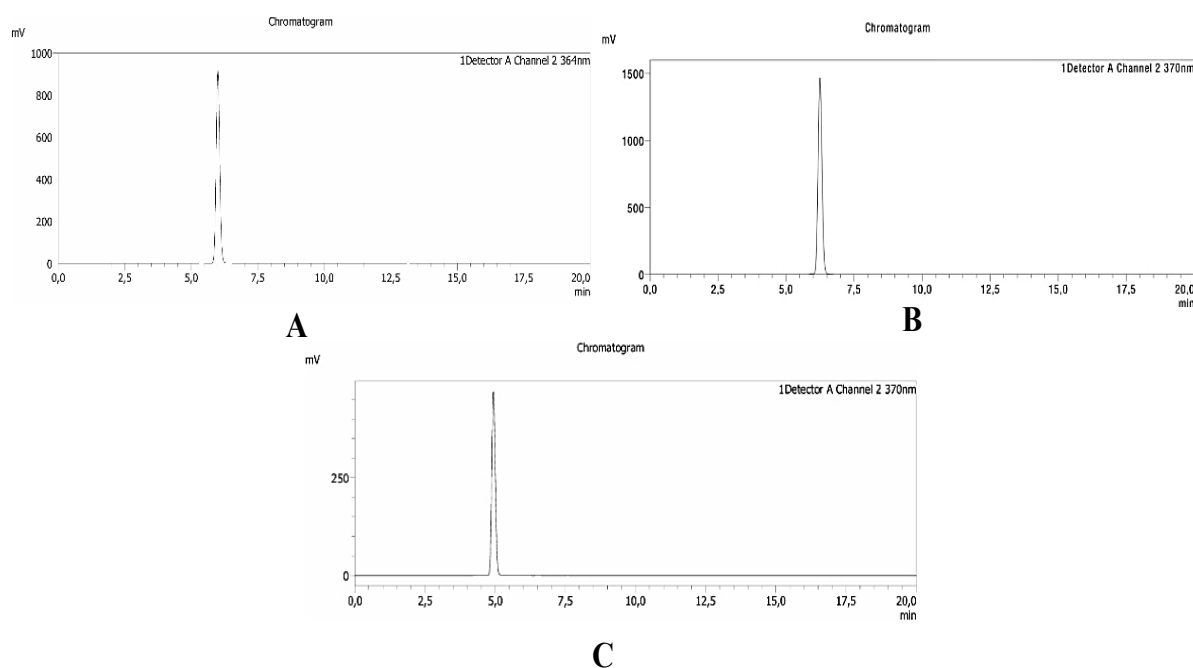


Figure 3. Chromatogram peak of HPLC of the compounds A, B, and C

(4.937 minutes) is shorter than that for A (6.002 minutes) and B (6.245 minutes), which is consistent with their polarity. All three compounds have a narrow melting point range ($\leq 2^\circ\text{C}$) which is a characteristic of pure compounds^[24]. The presence of impurities will widen the melting point range of a compound^[25].

The characterisation of the structures of compounds A, B, and C was performed using UV-

in compound C is consistent with the presence of three electron-donating groups. The presence of auxochromic groups such as alkoxy (OCH_3) will increase the intensity of absorption and shift band I towards the bathochromic region (red shift). FT-IR spectrum analysis in **Table 1**, which has similar characteristics, in all three compounds, as their functional groups are the same. The bands at 3463–3228, 3421–3347 and 3423–3347 cm^{-1} correspond to the N–H stretching

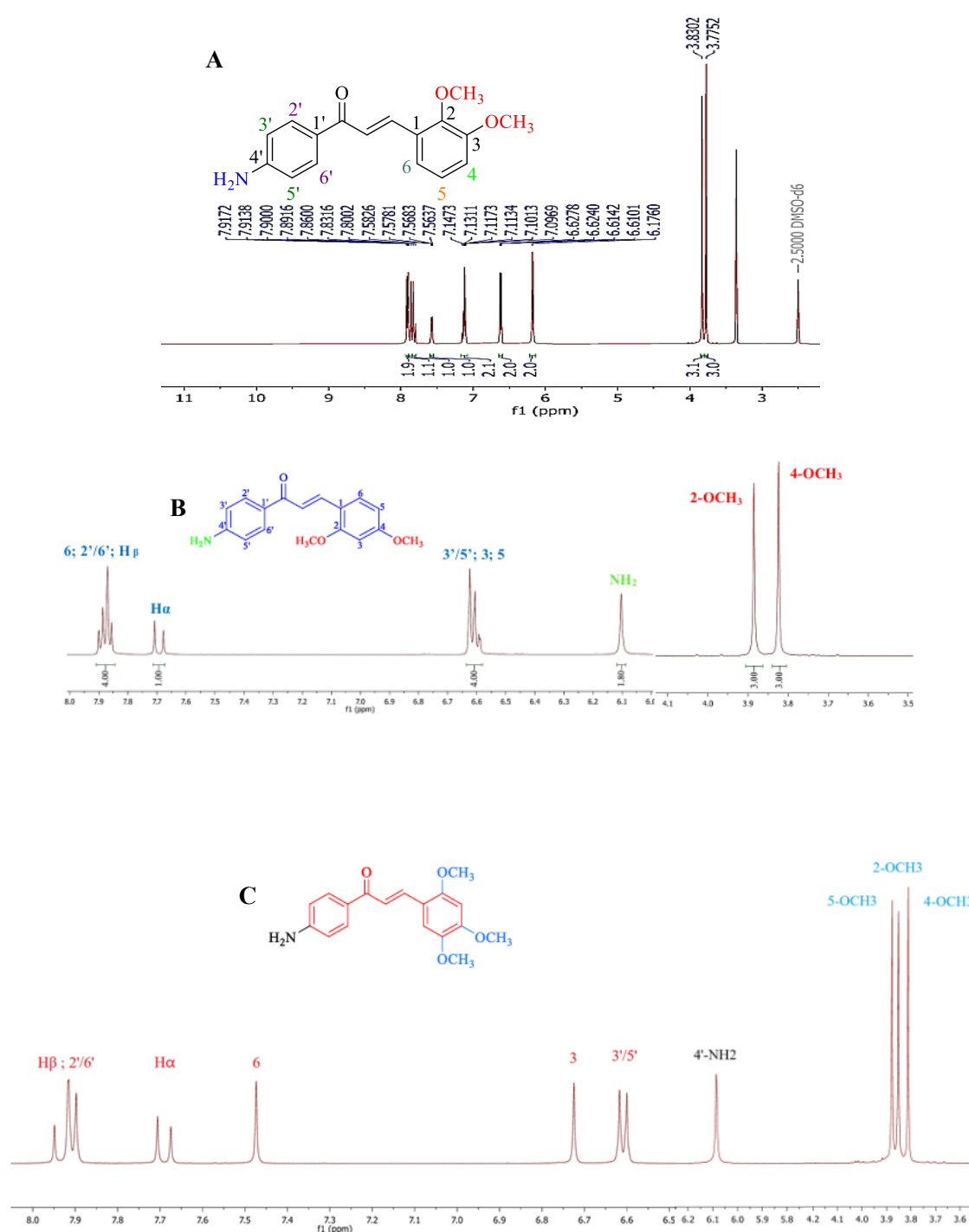


Figure 4. The structures and atomic numbering of the compounds (A) 4'-amino-2,3-dimethoxychalcone, (B) 4'-amino-2,4-dimethoxychalcone, and (C) 4'-amino-2,4,5-trimethoxychalcone, as a reference for the ¹H-NMR signal labelling in Table 2.

vibrations of the primary amino group (range 3500–3300 cm⁻¹). Methoxy C-H groups in the compound is seen at wave numbers 2834, 2836, 2830 cm⁻¹ (range 2850–2815 cm⁻¹)^[27]. The C=O ketone group is seen at wave numbers 1629, 1633,

1633 cm⁻¹ (range 1650–11600 cm⁻¹)^[27]. The aromatic C=C group is observed at wavenumbers 1598, 1594, 1594 cm⁻¹ (range 1615–1580 cm⁻¹)^[27].

Table 2. Interpretation of ^1H -NMR spectroscopic data of compounds A, B, and C

Atomic numbering	Chemical shift δ_{H} (ppm)		
	A	B	C
1	-	-	-
2	3,83 (s, 3H)	3,89 (s, 3H)	3,85 (s, 3H, -OCH ₃)
3	3,77 (s, 3H)	6,61 (m, 1H)	6,72 (s, 1H)
4	7,57 (d, 1H, J=7,15 Hz)	3,82 (s, 3H)	3,81 (s, 3H, -OCH ₃)
5	7,11(d, 1H, J=8,1Hz)	6,59 (d,1H, J= 2,5 Hz)	3,88 (s, 3H, -OCH ₃)
6	7,14 (d, 1H, J= 8,1 Hz)	7,89 (s, 1H, J= 6,5 Hz)	7,47 (s, 1H)
H α	7,82 (d, 1H, J= 15,7 Hz)	7,69 (d, 1H, J=15,5 Hz)	7,69 (d, 1H, J = 15,6 Hz)
H β	7,87 (d, 1H, J= 15,8 Hz)	7,89 (d, 1H, J=15,5 Hz)	7,93 (m, 1H, J = 15,65 Hz)
1'	-	-	-
2'/6'	7,91 (d, 2H, J= 8,6 Hz)	7,88 (d, 2H, J= 9 Hz)	7,91 (d, 2H, J = 8,5 Hz)
3'/5'	6,62 (d, 2H, J= 6,9 Hz)	6,62 (m, 2H, J= 10 Hz)	6,61 (d, 2H, J = 8,65 Hz)
4'	6,17 (s, 2H)	6,10 (s, 2H)	6,08 (s, 2H, -NH ₂)

As the FT-IR spectra of the three isomers are similar, the position of the methoxy group on ring B cannot be distinguished from the FT-IR data alone and was determined by ^1H -NMR.

Through ^1H -NMR measurements in **Table 2**, it was found that spectrum A and B have 17 protons ($\text{C}_{17}\text{H}_{17}\text{NO}_3$), whilst spectrum C has 19 protons ($\text{C}_{18}\text{H}_{19}\text{NO}_4$). A specific characteristic of the ^1H -NMR spectrum in chalcone compounds is the formation of a roofing signal, which indicates the presence of H α and H β in compounds A, B and C, which have a coupling constant (J) value of 15,6-15,8 Hz. In general, chalcone compounds exhibit α and β proton signals with a trans configuration and a coupling constant of 15-16 Hz^[28]. In addition, other characteristic signals are shown by the amino group (NH_2) detected at chemical shifts of 6,17; 6,10; 6,08 ppm, which indicate singlet signals (2H). The characteristic of the methoxy group is shown at chemical shifts ranging from 3,77-3,89 ppm (s, 3H). From the above information and the presence of this coupling value may indicate the existence of a core-core bonding environment within the structure of the target compound; in this case, the coupling constant supporting the trans

configuration of the synthesised chalcone compound.

The structure of the synthesised chalcone was confirmed by a combination of UV-Vis, FT-IR and ^1H -NMR analyses. The UV-Vis spectrum indicated the presence of a conjugated π -electron system, whilst the FT-IR spectrum confirmed the presence of a conjugated carbonyl group and functional groups characteristic of the target compound, such as NH_2 and methoxy group. Furthermore, the ^1H -NMR spectrum provides evidence of aromatic and α,β -unsaturated protons, as well as signals indicating the presence of NH_2 and methoxy groups at specific shifts. It can be concluded that the three compounds have molecular structures and functional groups that are consistent with the target compounds.

Antimalarial Activity Test

Antimalarial activity was conducted in vitro by analyzing the ability of compounds to inhibit the polymerization of heme into β -hematin as an indicator of compound effectiveness^[29]. This study is a cell-free model of the parasite's heme detoxification process; as such, it only demonstrates the inhibition of β -haematin formation and does not reflect the compound's full mechanism of

Table 3. In vitro antimalarial activities of compounds A, B, C, and positive control

No	Compounds	Concentration ($\mu\text{g/mL}$)	Inhibition of β -hematin (%)	IC ₅₀	
				$\mu\text{g/mL}$	μM
1	A	150	74,70	107,30	378,71
		125	58,33		
		100	42,75		
		75	30,56		
		50	25,00		
2	B	125	85,61	79,89	282
		100	69,51		
		75	44,15		
		50	29,02		
		25	1,22		
3	C	125	100,09	57,47	183,41
		62,5	50,44		
		31,25	33,10		
4	Positive Control (Hydroxychloroquine sulphate)	1000	99,76	187,02	430,9
		500	73,23		
		250	53,98		
		125	48,45		
		62,5	41,25		
		31,25	37,03		

action in whole parasites. Test results were measured using a microplate reader at wavelengths of 415 nm and 630 nm, referring to a modified method using Tween-20 as an initiator^[3]. Measurements at different wavelengths were performed to determine the absorbance value of hemin chloride (415 nm) and β -hematin (630 nm). Incubation was carried out for 250 minutes at a temperature of 37°C^[21].

The test was conducted on synthesized compounds and positive controls in various concentrations with three repetitions, and the values obtained were plotted to obtain a linear regression equation and determine the IC₅₀ value, which is the concentration that can inhibit 50% of heme polymerization into β -hematin. The test results can be seen in **Table 3** by comparing the IC₅₀ values between the chalcone derivatives and positive control, which are expressed in $\mu\text{g/mL}$

and converted to μM due to good selectivity referring to the classification of^[30].

The results show that compound C has the lowest IC₅₀ value at 183,41 μM , followed by compound A at 282 μM ; B at 378,71 μM ; and the positive control at 430,9 μM . Suwito et al., through a molecular docking study, reported that the amino group in chalcone plays a key role in enhancing its antimalarial potential by providing electrostatic interaction with amino acid residues in the target Plasmodium protein^[31]. In this study, the different positions of the methoxy group in each chalcone derivative compound affect its mechanism in inhibiting the formation of β -hematin. Compound C (three methoxy groups) is more active than B and A (two methoxy groups), and B (2,4-dimethoxy) is more active than A (2,3-dimethoxy). Compounds A and B have the same molecular formula and

molecular mass and differ only in the position of one methoxy group (position 3 in A and position 4 in B); therefore, the difference in their activity indicates that the position of the substituent has a significant effect. This result shows that variations in substitution on the B ring can affect the activity of chalcone as an antimalarial agent^[32].

Conclusions

Based on the results of the study, it can be concluded that amino methoxy chalcone derivatives synthesised through the Claisen-Schmidt reaction yielded 50,19%, 31,18% and 19,42%. In vitro activity testing showed antimalarial activity with a mechanism of heme polymerisation inhibition. 4'-amino-2,4,5-trimethoxy chalcone with methoxy substitution at positions 2,4,5 on ring B showed the highest antimalarial activity. However, the scope of this study is limited to the inhibition of heme polymerisation into B-haematin; therefore, further research is required to establish the direct in vitro antimalarial activity of these compounds against *P. falciparum* and to confirm biological relevance. These results have the potential as a basis for further research to support the development of antimalarial drug design in the future.

Acknowledgments

The authors would like to thank STIFAR Riau for providing facilities for this research, as well as the Integrated Chemistry Laboratory of FMIPA (ITB) for permission to use their equipment.

Declaration of Generative AI and AI-assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

Investigation, R.D.; writing-original draft, R.D.; Supervision, I.I. M.O. and E.M.; data analysis, C.F.Y. L.M. and P.J.; reviewed the manuscript, all authors.

Funding

This research received no external funding.

Declaration of Conflicts of Interest

The authors declare no conflict of interest.

References

1. World Health Organization. *World Malaria Report 2021*. World Health Organization, Geneva (2021).
2. World Health Organization. *World Malaria Report 2023*. World Health Organization, Geneva (2023).
3. Huy, N.T., Uyen, D.T., Maeda, A., Trang, D.T.X., Oida, T., Harada, S. and Kamei, K. Simple colorimetric inhibition assay of heme crystallization for high-throughput screening of antimalarial compounds. *Antimicrob. Agents Chemother.* 51: 350-353 (2007). <https://doi.org/10.1128/AAC.00985-06>.
4. Muti'ah, R. Penyakit malaria dan mekanisme kerja obat-obat antimalaria. *Al-chemy.* 2: 80-91 (2013).
5. Rahmasari, F.V., Asih, P.B.S., Rozi, I.E., Wangsamuda, S., Risandi, R., Dewayanti, F.K., Permana, D.H. et al. Evolution of genetic markers for drug resistance after the introduction of dihydroartemisinin-piperaquine as first-line anti-malarial treatment for uncomplicated falciparum malaria in Indonesia. *Malar. J.* 22: 1-13 (2023).
6. Kementerian Kesehatan Republik Indonesia. *Keputusan Menteri Kesehatan Republik Indonesia tentang Pedoman Nasional Pelayanan Kedokteran Tata Laksana Malaria*. Menteri Kesehatan Republik Indonesia (2019).
7. Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyto, A.P., Tarning, J. et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* 361: 455-467 (2009).
8. Sinha, S., Medhi, B., Radotra, B.D., Batovska, D.I., Markova, N., Bhalla, A. and Sehgal, R. Antimalarial and immunomodulatory potential of chalcone derivatives in experimental model of malaria. *BMC Complement. Med. Ther.* 22: 1-14 (2022).
9. Ridley, R.G. Medical need, scientific opportunity and the drive for antimalarial drugs. *Nature.* 415: 686-693 (2002).

10. Afshar, F., Delazar, A., Asnaashari, S., Vaez, H., Zolali, E. and Asgharian, P. Screening of anti-malarial activity of different extracts obtained from three species of *Scrophularia* growing in Iran. *J. Pharm. Res.* 17: 668-676 (2018).
11. Ouyang, Y., Li, J., Chen, X., Fu, X., Sun, S. and Wu, Q. Chalcone derivatives: role in anticancer therapy. *Biomolecules.* 11: 894 (2021).
12. Mezgebe, K., Melaku, Y. and Mulugeta, E. Synthesis and pharmacological activities of chalcone and its derivatives bearing N-heterocyclic scaffolds: a review. *ACS Omega.* 8: 19194-19211 (2023).
13. Chen, Y.H., Wang, W.H., Wang, Y.H., Lin, Z.Y., Wen, C.C. and Chern, C.Y. Evaluation of the anti-inflammatory effect of chalcone and chalcone analogues in a zebrafish model. *Molecules.* 18: 2052-2060 (2013).
14. Goyal, K., Kaur, R., Goyal, A. and Awasthi, R. Chalcones: a review on synthesis and pharmacological activities. *J. Appl. Pharm. Sci.* 11(Suppl.1): 1-14 (2021).
15. Lal, K., Yadav, P., Kumar, A., Kumar, A. and Paul, A.K. Design, synthesis, characterization, antimicrobial evaluation and molecular modeling studies of some dehydroacetic acid-chalcone-1,2,3-triazole hybrids. *Bioorg. Chem.* 77: 236-244 (2018).
16. Shukla, P., Satyanarayana, M., Verma, P.C., Tiwari, J., Dwivedi, A.P., Srivastava, R., Rehuja, N. et al. Chalcone-based aryloxypropanolamine as a potential antidiabetic and antidiyslipidaemic agent. *Curr. Sci.* 112: 1675-1689 (2017).
17. Syahri, J., Nasution, H., Nurohmah, B.A., Purwono, B. and Yuanita, E. Novel amino-alkylated chalcone: synthesis, biological evaluation, and docking simulation as potent antimalarial agents. *J. Appl. Pharm. Sci.* 10: 001-005 (2020).
18. Syahri, J., Yuanita, E., Nurohmah, B.A., Armunanto, R. and Purwono, B. Chalcone analogue as potent anti-malarial compounds against *Plasmodium falciparum*: synthesis, biological evaluation, and docking simulation study. *Asian Pac. J. Trop. Biomed.* 7: 675-679 (2017).
19. Suwito, H., Jumina, Mustofa and Kristanti, A.N.N. Chalcones: synthesis, structure diversity and pharmacological aspects. *J. Chem. Pharm. Res.* 6: 1076-1088 (2014).
20. Setyarini, I.I. and Astuti, I. *Antiplasmodial Activity and Selectivity of Chalcone Derivatives*. Universitas Gadjah Mada, Yogyakarta (2015).
21. Dona, R., Frimayanti, N., Jasril and Hendra, R. Synthesis and in vitro antimalarial activity of amino chalcone derivative compounds by inhibition of heme polymerization. *J. Sains Farm. Klin.* 11: 127-135 (2024).
22. Fajrin, A.V. *Sintesis dan Uji Aktivitas Turunan Kalkon Berbahan Dasar 2,4-Dimetoksiasetofenon sebagai Senyawa Antimalaria*. Skripsi, Universitas Gadjah Mada, Yogyakarta (2022).
23. Maharani, L. *Sintesis Senyawa 4'-Amino-2,4-Dimetoksikalkon dan Uji Aktivitas Antimalaria secara In Vitro*. Skripsi, Sekolah Tinggi Ilmu Farmasi Riau, Pekanbaru (2025).
24. Suryelita, Etika, S.B. and Kurnia, N.S. Isolasi dan karakterisasi senyawa steroid dari daun cemara natal (*Cupressus funebris* Endl.). *Eksakta.* 18: 86-94 (2017).
25. Cahyono, B. and Suzery, M. *Metode Pemisahan Bahan Alam: Aspek Teoritis dan Eksperimen*. PT Kompas Ilmu, Jakarta (2018).
26. Pratiwi, D.N., Utami, N. and Pratimasari, D. Characterization and determination of total flavonoid content of extract and fraction of papaya jantan flower (*Carica papaya* L.) using UV-Vis spectrophotometry. *J. Ilm. Farm.* 18: 219-233 (2022).
27. Nandiyanto, A.B.D., Oktiani, R. and Ragadhita, R. How to read and interpret FTIR spectroscopy of organic material. *Indones. J. Sci. Technol.* 4: 97-118 (2019).
28. Syah, Y.M. *Dasar-Dasar Penentuan Struktur Molekul Berdasarkan Data Spektrum ¹H & ¹³C NMR*. Perpustakaan Nasional RI (2016).
29. Septiana, E., Umaroh, A., Gangga, E. and Simanjuntak, P. Aktivitas penghambatan polimerisasi heme ekstrak daun sembung (*Blumea balsamifera*) sebagai antimalaria. *Bul. Penelit. Tanam. Rempah dan Obat.* 28: 29-36 (2017).

-
30. Batista, R., De Jesus Silva Júnior, A. and De Oliveira, A.B. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. *Molecules*. 14: 3037-3072 (2009).
31. Suwito, H., Jumina, Mustofa, Pudjiastuti, P., Fanani, M.Z., Kimata-Arigo, Y., Katurahira, R. et al. Design and synthesis of chalcone derivatives as inhibitors of the ferredoxin-ferredoxin-NADP⁺ reductase interaction of *Plasmodium falciparum*: pursuing new antimalarial agents. *Molecules*. 19: 21473-21488 (2014). <https://doi.org/10.3390/molecules191221473>.
32. Sinha, S., Batovska, D.I., Medhi, B., Radotra, B.D., Bhalla, A., Markova, N. and Sehgal, R. In vitro anti-malarial efficacy of chalcones: cytotoxicity profile, mechanism of action and their effect on erythrocytes. *Malar. J.* 18 (2019). <https://doi.org/10.1186/s12936-019-3060-z>.