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Isolation, structure elucidation, and synthesis of antiplasmodial quinolones from *Crinum firmifolium*

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Abstract

Antiplasmodial bioassay guided fractionation of a Madagascar collection of *Crinum firmifolium* led to the isolation of seven compounds. Five of the seven compounds were determined to be 2-alkylquinolin-4(1*H*)-ones with varying side chains. Compounds **1** and **4** were determined to be known compounds with reported antiplasmodial activities, while **5** was believed to be a new branched 2-alkylquinolin-4(1*H*)-one, however, it was isolated in limited quantities and in admixture and therefore was synthesized to confirm its structure as a new antiplasmodial compound. Along with **5**, two other new and branched compounds **6** and **7** were synthesized as well. Accompanying the five quinolones were two known compounds **2** and **3** which are inactive against *Plasmodium falciparum*. The isolation, structure elucidation, total synthesis, and biological evaluation of these compounds are discussed in this article.

Graphical abstract

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Note

The authors declare no competing financial interests.

Supplementary Material

Supplementary material for compounds **1** – **22** have been provided including ¹H NMR, ¹³C NMR, HSQC, HMBC, HRESIMS and GC-MS data.

Keywords

Antiplasmodial; Quinolone; *Crinum*; Amaryllidaceae

1. Introduction

The genus *Crinum* (Amaryllidaceae) contains around 110 accepted species with over 270 synonyms.¹ Members of this genus possess large extravagant flowers on leafless stems and are distributed in moist sites, such as forests, river edges, seasonal pools, or salt pans, and can be found throughout the tropics of Africa, Asia, America, Southern Africa, Madagascar, and the Mascarene and Pacific Islands.² Extracts from *Crinum* species have been used traditionally to treat a variety of ailments including fever, pain management, swelling, sores and wounds, cancer, and malaria.³ The genus is a rich source of norbelladine type alkaloids such as lycorine, crinine, and narciclasine alkaloids.⁴ Alkaloids isolated from various *Crinum* species have shown activity in a wide variety of assays including analgesic, anticancer, antibacterial, antiviral, antifungal, and antimalarial assays.³

Malaria is a vector-borne disease that has a disproportionate effect in poor and underdeveloped countries without access to western medicine, and with the appearance of artemisinin-resistant parasites in 2008 there is an urgent need for new and affordable medicines.⁵ Since plants have been successful in the past with producing antimalarial medicines such as quinine and artemisinin, our group is investigating plant extracts from the Natural Products Discovery Institute (NPDI) collection for new compounds or known compounds with new activity against the malaria parasite *Plasmodium falciparum*. Recently, our group has had success with isolating new potentially antiplasmodial compounds from an extract of *Crinum erubescens* L. f. ex Aiton.⁶ This prompted us to investigate the *Crinum* species *C. firmifolium* in our International Cooperative Biodiversity Group (ICBG) collection. Its extract was found to possess weak activity against *P. falciparum* ($IC_{50} \sim 10 \mu\text{g/mL}$), which had increased activity in the mid-polarity fraction after a simple liquid-liquid extraction ($IC_{50} 5 < x < 10 \mu\text{g/mL}$). It was thus selected for further investigation.

2. Results and Discussion

2.1. Isolation

The ethanolic extract of *C. firmifolium* (2.1 g) was dissolved in aqueous methanol and partitioned with hexanes. The aqueous methanol fraction was then dried and suspended in water and exhaustively extracted with EtOAc to afford an active EtOAc fraction (286 mg, $IC_{50} 5 < x < 10 \mu\text{g/mL}$). The EtOAc fraction was separated further into four fractions via a C_{18} solid phase extraction (SPE) with different concentrations of aqueous methanol. The second fraction (127 mg) was found to be active ($IC_{50} \sim 1.25 \mu\text{g/mL}$) and was separated further into the ten fractions H1 – H10 by C_{18} HPLC using an aqueous acetonitrile solvent

system. Fraction H2 was found to have potent antiplasmodial activity ($IC_{50} \lll 1.25 \mu\text{g/mL}$), but after multiple attempts utilizing a variety of stationary and mobile phases only compound **1** could only be isolated, and then only as the major component of fraction H2-4 containing a mixture of similar compounds in a yield of 0.2 mg. The weakly active fraction H3 ($IC_{50} 1.25 < \times < 2.5 \mu\text{g/mL}$) was subjected to HPLC separation on a C_{18} column using aqueous MeOH, but only the two inactive compounds **2** and **3** could be isolated; no active compound was obtained from this fraction. Fraction H4 ($IC_{50} \lll 1.25 \mu\text{g/mL}$) was separated into the ten fractions H4-1 – H4-10 by HPLC on a C_{18} column with elution by aqueous MeOH. Fractions H4-6 and H4-8 were both found to be highly active ($IC_{50} \lll 1.25 \mu\text{g/mL}$) but the only compound that could be isolated from fraction H4-6 was **4** as the major component of a mixture of similar compounds, and likewise compound **5** was the only compound obtained from fraction H4-8 as the major component of a mixture of similar compounds. The small amount of available extract prevented additional isolation studies.

2.2. Structure Elucidation

The major component of H2-4 was determined to have the composition $C_{16}H_{21}NO$ from its HRESIMS. Its ^1H NMR spectrum in CD_3OD showed strong signals for an aromatic moiety with four protons at δ_{H} 8.21 (dd, $J = 8.2, 1.5 \text{ Hz}$, 1H), δ_{H} 7.70 (ddd, $J = 8.4, 7.0, 1.5 \text{ Hz}$, 1H), δ_{H} 7.58 (brd, $J = 8.4 \text{ Hz}$, 1H), and δ_{H} 7.40 (ddd, $J = 8.2, 7.0, 1.0 \text{ Hz}$, 1H) indicating the presence of a 1,2-disubstituted aromatic ring. A single isolated α -proton was observed at δ_{H} 6.24 (s, 1H). In the aliphatic region of the spectrum, a triplet-like multiplet at δ_{H} 2.78 – 2.65 (m, 2H) along with a pentet at δ_{H} 1.77 (p, $J = 7.8 \text{ Hz}$, 2H), a broad signal at δ_{H} 1.49 – 1.24 (m, 8H), and a triplet at δ_{H} 0.90 (t, $J = 6.9 \text{ Hz}$, 3H) all suggested the presence of an *n*-heptyl alkyl chain. Comparison of literature data for compounds with the observed chemical formula and ^1H NMR signals identified the major component of H2-4 as the known antiplasmodial compound 2-heptylquinolin-4(1H)-one (HHQ) (**1**, Fig. 1).⁷ The major peaks in the ^1H NMR spectrum of H2-4 in CD_3OD matched those of an authentic sample of HHQ.

Compound **2** (0.3 mg) was found to have the composition $C_{15}H_{15}NO_3$ from its HRESIMS. The spectrum also displayed a very stable fragment resulting from the loss of water, indicating the presence of a hydroxyl group. Two geminally coupled benzylic protons were observed in its ^1H NMR spectrum at δ_{H} 4.28 (d, $J = 12.0 \text{ Hz}$, 1H) and δ_{H} 4.21 (d, $J = 12.0 \text{ Hz}$, 1H), consistent with the protons of a benzyl alcohol. The most shielded resonance observed in its ^1H NMR spectrum was at δ_{H} 2.75 (s, 3H) for an N-methyl group. A methylenedioxy resonance was observed at δ_{H} 6.01 (s, 2H) along with two singlet aromatic protons at δ_{H} 7.03 (s, 1H) and δ_{H} 6.69 (s, 1H) which could indicate the presence of a 2,3,5,6-substituted aromatic ring. The remaining four aromatic protons made up a 2,3-substituted aromatic coupling pattern resulting from two doublet-like proton resonances at δ_{H} 7.00 and δ_{H} 6.75 and two triplet-like proton resonances at δ_{H} 7.31, and δ_{H} 6.84, which support this claim. The identity of the isolated compound was determined to be the known compound **2** based on a comparison with literature data.⁸

Compound **3** (0.4 mg) was found to have the composition $C_{16}H_{11}NO_3$ from its HRESIMS. Examining the ^1H NMR spectrum revealed a signal for a methoxy methyl group at δ_{H} 4.10 (s, 3H) as the most shielded proton resonance. The remaining observable signals were all

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deshielded aromatic proton signals. Two coupled methine pyrrole-like double bond resonances at δ_{H} 8.06 (d, $J = 3.6$ Hz, 1H) and δ_{H} 6.91 (d, $J = 3.6$ Hz, 1H) could be observed, as well two singlets at δ_{H} 8.04 (s, 1H) and δ_{H} 7.79 (s, Hz, 1H), indicating a 2,3,5,6-substituted aromatic ring. The remaining aromatic resonances were all coupled to one another with resonances at δ_{H} 7.96 (d, $J = 7.7$ Hz, 1H), δ_{H} 7.75 (d, $J = 7.7$ Hz, 1H) and δ_{H} 7.49 (t, $J = 7.7$ Hz, 1H), indicating a 4,5,6-substituted aromatic ring moiety. A search of the literature revealed two possible structures that fit the observed data, with the difference being the location of the methoxy group. Both possible isomers of the compound have been isolated, and the methoxy groups, as well as the singlets for positions H-8 and H-11, all have differing chemical shifts in the two isomers.⁹ The resonances observed in the isolated molecule agree with the published literature for a methoxy methyl in position 10 and confirm the structure of the isolated compound to be that of pratorinine (**3**).¹⁰

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The major compound from fraction H4-6 (0.2 mg) was approximately 76% pure as judged by its ^1H NMR spectrum. It had the composition $\text{C}_{18}\text{H}_{23}\text{NO}$ as deduced from its HRESIMS, and its ^1H NMR spectrum showed signals for an alkenyl chain, with vinyl proton signals at δ_{H} 5.45 – 5.39 (m, 2H) and signals for the same 2-alkylquinolin-4(1*H*)-one moiety as in **1**. Comparison of these data with literature data identified the compound as the known compound acutine (**4**), or 2-[(4*Z*)hept-4-en-1-yl]quinolin-4(1*H*)-one.¹¹ Acutine has been reported to have moderate antiparasmodial activity against the chloroquine-sensitive 3D7 strain of *P. falciparum*.¹¹

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The major compound from fraction H4-8 (approximately 50% by ^1H NMR spectroscopy) was also isolated in very limited quantities (0.2 mg). Its HRESIMS data indicated the chemical composition $\text{C}_{17}\text{H}_{23}\text{NO}$, and its ^1H NMR spectrum showed signals for the same 2-alkylquinolin-4(1*H*)-one moiety observed in **1** and **4**. Examination of the aliphatic region of the spectrum revealed a methylene multiplet at δ_{H} 2.73 (m, 2H) and a 6H methyl doublet at δ_{H} 0.88 (d, $J = 6.6$ Hz) that could be clearly distinguished from signals of the other components; these signals indicated the presence of a 6'-methylheptyl chain. The only compound in the literature with the chemical formula $\text{C}_{17}\text{H}_{23}\text{NO}$ containing a 2-alkylquinolin-4(1*H*)-one moiety is 2-octylquinolin-4(1*H*)-one (**8**), so the major compound from fraction H4-8 must be a new compound with the structure **5** (Fig. 1). Since **5** could not be isolated in pure form, its structure and biological activity were confirmed by synthesis. The new analogues **6** and **7** and the known compound **8** were also prepared for determination of basic structure-activity relationships.

2.3. Synthetic Studies

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There are many synthetic routes in the literature for 2-alkylquinolin-4(1*H*)-ones. The published procedures include the Conrad – Limpach synthesis,¹² the InCl_3 – mediated solvent free Conrad – Limpach reaction,¹³ and the microwave cyclization of β – enaminoates,¹⁴ but none of these afforded the desired 2-alkylquinolin-4(1*H*)-ones in our hands. However, InCl_3 was found to rapidly catalyze the formation of the intermediate β – enaminoates from aniline and the corresponding β – ketoesters,¹⁵ and cyclization of the β – enaminoates to afford the desired 2-alkylquinolin-4(1*H*)-ones was finally achieved with polyphosphoric acid.¹⁶

The long chain β – ketoesters needed for the synthesis of the β – enaminates were synthesized via alkylation of ethyl acetoacetate with the corresponding alkyl iodides.¹⁷ Alkyl iodides **14** – **16** were synthesized by standard routes starting from terminal alcohols **11** – **13**; iodides **14** and **15** were obtained as racemates. Alcohols **12** and **13** were prepared from terminal alkenes **9** and **10** by reaction with borane THF solution and hydrogen peroxide to afford the corresponding primary alcohols, and alcohol **11** was purchased. These three primary alcohols were reacted with triphenyl phosphine, imidazole, and iodine to afford the primary alkyl iodides **14**, **15**, and **16**, and primary alkyl iodide **17** was purchased. The dianion of ethyl acetoacetate **18** was produced with two equivalents of LDA and then alkylated with halides **14** – **17** to prepare the desired long chain ketoesters **19** – **22** in low to moderate yield. Condensation reactions were then carried out with aniline, InCl_3 , and **19** – **22** to afford the desired β – enaminates, which were cyclized with polyphosphoric acid without isolation to afford the 2-alkylquinolin-4(1*H*)-one title compounds **5** – **8** (Scheme 1).

2.4. Analysis

Once the total syntheses of **5** – **8** were complete and their structures were confirmed via NMR and HRESIMS, the identities of the isolated natural product could finally be determined. The first analysis conducted was a ^1H NMR comparison of the major compound from fraction H4-8 with that of compound **5** to determine if they were in fact the same compound. Comparison of the aromatic protons and the alpha proton were an exact match, and comparison of the aliphatic methylene and methyl proton signals for 1',6' – Me, and 7' were also an exact match (Fig. 2). Comparison of the ^1H NMR spectra of **5** – **8** with that of the major compound from fraction H4-8 clearly indicated that among quinolones with the composition $\text{C}_{17}\text{H}_{23}\text{NO}$, synthetic **5** is the best match to the spectrum of the isolated compound (Table 1 and Figure 2).

The next comparison conducted was an HPLC analysis. The method used for the isolation of the natural products did not give baseline separation for all four synthetic compounds, so a new method was developed utilizing a C_{12} column with aqueous MeCN with 0.1% TFA. This method was also able to separate the impurity from the quinolones isolated from the natural source. The flow rate of 0.3 mL/min was so slow that very subtle differences could be observed in retention times (t_R in min) on repeat injections of the same compound. To help account for the variance in retention times the natural product was “bracketed” with two runs of the synthetic compound with one before and the other after the natural product run. Comparison of the retention times for the synthetic compound were t_R 35.49 min for the run before and t_R 35.81 min for the run after the natural product had been run, while the natural product was found to elute at t_R 35.93 min. Although these retention times are not identical, this appears to be due to small random variations in pumping speed. The combination of identical key resonances in their ^1H NMR spectra and identical HPLC retention times within experimental error assures that the isolated and synthetic compounds have the same structure.

2.5. Bioassay Data

The isolated compounds **2** and **3** and the synthetic compounds **1** and **5** – **8** were tested against the chloroquine-resistant Dd2 strain of *P. falciparum*. Compound **4** was not obtained

completely pure, so accurate determination of its antiplasmodial activity was not possible. However, based on the activity of the fraction H4-6 which contained **4** as its major component, it almost certainly is active against the chloroquine-resistant Dd2 strain of *P. falciparum*. It has been previously shown to have activity against the chloroquine-sensitive 3D7 strain of *P. falciparum*.¹¹

Compound **1** was found to have a modest IC₅₀ against the Dd2 strain of *P. falciparum* of $1.3 \pm 0.3 \mu\text{M}$; it has also been reported to have activity against the W2 and D6 strains of *P. falciparum*.⁷ Compounds **2** and **3** were found to be inactive against the Dd2 strain of *P. falciparum* at the highest concentrations tested of $39 \mu\text{M}$ and $38 \mu\text{M}$ respectively. The C₁₇H₂₃NO isomers **5**, **6**, **7**, and **8** had IC₅₀ values against the Dd2 strain of *P. falciparum* of $583 \pm 330 \text{ nM}$, $330 \pm 43 \text{ nM}$, $750 \pm 93 \text{ nM}$, and $3.7 \pm 0.5 \mu\text{M}$ respectively. The quinolones were also tested against the chloroquine-sensitive 3D7 strain of *P. falciparum*. Compounds **1**, **5**, **6**, **7**, and **8** had IC₅₀ values of $1.3 \pm 0.3 \mu\text{M}$, $501 \pm 82 \text{ nM}$, $385 \pm 78 \text{ nM}$, $921 \pm 206 \text{ nM}$, and $3.8 \pm 0.6 \mu\text{M}$ respectively. The compounds were also tested against the A2780 drug-sensitive mammalian cancer cell line. Alkaloids **2** and **3** were found to be inactive at all concentrations tested, with IC₅₀ values greater than $78 \mu\text{M}$ and $75 \mu\text{M}$, respectively. The quinolones **1**, **5**, **6**, **7**, and **8** had IC₅₀ values of 35 ± 5 , 34 ± 5 , 29 ± 1 , 8.7 ± 2.1 , and $12 \pm 2 \mu\text{M}$ activities, respectively (Table 2).

The availability of quinolones **1**, **5**, **6**, **7**, and **8** enabled some preliminary structure-activity relationships to be noted. The first observation is that the potency of these compounds against the drug-resistant Dd2 strain of *P. falciparum* was approximately the same as their potency against the drug-sensitive 3D7 strain. The next observation is that shorter side chains appear to give more potent activity than longer ones. Thus compounds **1** and **5** – **7**, all with *n*-heptyl or methylated *n*-heptyl side chains, are all more potent by a factor of three or more than compound **8** with an *n*-octyl side chain. This observation suggests that further increases in the length of the side chain would be unfavorable for bioactivity. A final observation is that chain branching improves activity. Compounds **1**, **5**, **6**, and **7** all possess a heptyl side chain, and the branched chain compounds **5**, **6**, and **7** all showed improved activities over the *n*-heptyl side chain of **1**.

The selectivity of the quinolones can be measured via a selectivity ratio (A2780 IC₅₀ value/Dd2 IC₅₀ value) that is related to a therapeutic index. Artemisinin has been reported to have a therapeutic index of 4987, and is considered to be nontoxic by most researchers.¹⁸ Compounds **1**, **5**, **6**, **7**, and **8** have selectivity ratios of 26.6, 57.7, 89.0, 11.6, and 3.4, respectively. Although these values are not ideal, they do indicate that chain branching enhances selectivity for *P. falciparum* over A2780 mammalian cells, with compound **6** showing not only the best activity against the Dd2 strain of *P. falciparum* but also showing the largest selectivity ratio. It is thus possible that analogs with increased chain branching might have better antiplasmodial activity and improved selectivity.

3. Conclusions

The genus *Crinum* continues to produce new and biologically active alkaloids. Four known alkaloids (**1** – **4**) were isolated along with the new alkaloid **5**, whose structure and biological

activity was confirmed through total synthesis. Unfortunately, the small amounts and difficulty of separation of the other two C₁₇H₂₃NO quinolones that could be identified prevented their structural assignment via total synthesis or spectroscopic comparison. Along with the synthesis of **5** the other new natural product-like quinolones **6** and **7** were successfully synthesized and biologically evaluated. The isolated and synthesized quinolones display moderate levels of selectivity for *P. falciparum* over mammalian cells. At this time the quinolone moiety has been thoroughly investigated for malaria activity, with several different structure-activity relationships from studies of the effect of substituents at the 1, 2, and 3 positions, as well as modifying the electronics of the aromatic ring.^{19–21}

4. Experimental Section

4.1. General

All chemicals used were purchased from either Sigma-Aldrich or the Fisher Scientific Company and were used without further purification. 2-Heptylquinolin-4(1H)-one (HHQ) was purchased from Sigma-Aldrich. UV spectra were measured UV-1201 on a Shimadzu spectrophotometer. ¹H and ¹³C NMR spectra were obtained on a Bruker Avance 500 spectrometer in CD₃OD for solubility reasons. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS spectrometer and an Agilent 7890B GC system coupled with a 5977A MSD. Semipreparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector, a SCL-10A system controller, and a Phenomenex 5 μm, 100 Å, Luna C₁₈(2) (250 × 10 mm) column, a Kromasil 5 μm, 60 Å, Diol (250 × 10 mm) column, or a Phenomenex 4 μm, 90 Å, Jupiter (150 × 2 mm) column. Solid phase extractions were conducted with Thermo Scientific hypersep C₁₈ 500 mg in 3 mL SPE cartridges, and all reverse phase TLC was conducted with Sorbtech C₁₈-W silica TLC plates, w/UV254 on an aluminum backing with a thickness of 150 μm.

4.2. Plant material

Leaves of *Crinum firmifolium* Baker were collected in the dry deciduous forest at Solanampilana, 3 km south of the village of Befarafara, district of Vohémar, region of SAVA, Madagascar, at coordinates 13°05'42"S, 049°34'57"E on 10 Dec 2006 and an elevation of 137 m. The plant was herbaceous with a bulb 10 cm in diameter, leaves 65 cm, inflorescence 1 m high, with 1 to 2 inflorescences per bulb. It had white petals and red stamens and stalk. Duplicate voucher specimens (Rakotonandrasana 1053) were deposited at the Herbaria of the Centre National d'Application des Recherches Pharmaceutiques (CNARP), of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar (TAN), of the Muséum National d'Histoire Naturelle in Paris, France (P) and of the Missouri Botanical Garden, St. Louis, Missouri (MO).

4.3 Plant extraction

A ground sample of *C. firmifolium* leaves (43 g) was extracted with EtOH (700 mL) at room temperature to yield 4.12 g of crude EtOH extract designated MG 4166, and a 1.0 g portion of this extract was made available to Virginia Tech. Similar amounts were made available to Dow AgroSciences and Eisai Inc., who were kind enough to send their remaining supply to Virginia Tech for bioassay-guided isolation.

4.4. Antiplasmodial bioassay

Each fraction and isolated compound was tested against the chloroquine/mefloquine-resistant Dd2 strain and the chloroquine-sensitive 3D7 strain of *P. falciparum* in a 72-hour treatment assay using the malaria SYBR green I-based fluorescence assay as described previously.^{22–23} The antimalarial drug artemisinin was used as a positive control for the Dd2 and 3D7 strains of *P. falciparum*, with IC₅₀ values of 9.0 ± 2.7 nM and 5.9 ± 0.5 nM, respectively.

4.5. Antiproliferative bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Tech as previously reported.^{24–25} The A2780 cell line is a drug-sensitive ovarian cancer cell line.²⁶

4.6. Isolation of bioactive compounds

The EtOH extract of *C. firmifolium* (2.1 g) was dissolved in aqueous MeOH (95:5, 200mL) and extracted with hexanes (3 × 200 mL), which generated the inactive hexanes fraction (247 mg). The aqueous MeOH fraction was dried under reduced pressure and then suspended in water (200 mL) and extracted with EtOAc (3 × 200 mL). Both fractions were concentrated under reduced pressure, and the water fraction (1.4 g) was found to be inactive while the EtOAc fraction (286 mg) was found to possess modest activity (IC₅₀ 5 < × < 10 µg/mL). This fraction was separated further into four sub-fractions via a C₁₈ solid phase extraction (SPE) with different concentrations of aqueous methanol (20:80, 80:20, and 100:0) and a dichloromethane (DCM) flush. The second fraction (127 mg) was found to be active (IC₅₀ ~ 1.25 µg/mL) and was separated further into ten sub-fractions (H1 – H10) by C₁₈ HPLC with a H₂O/MeCN gradient starting from 62:38 to 30:70 over 40 min followed by a maintained flow at 0:100 for 20 min. Fraction H2 (3.4 mg, IC₅₀ value <<< 1.25 µg/mL) proved to be difficult to purify. The major active constituents were isolated as a mixture of similar compounds from diol HPLC with a H₂O/MeCN gradient starting from 70:30 to 63:37 over 20 min. This procedure afforded 0.2 mg of a mixture (Fraction H2-4) of similar compounds **1** (t_R 11.08 min) with an IC₅₀ value of <<< 1.25 µg/mL and an estimated purity of 66% as judged from its ¹H NMR spectrum. HPLC fraction H3 (2.5 mg) was found to be weakly active (IC₅₀ value 1.25 < × < 2.5 µg/mL) and was separated into eight fractions (H3-1 – H3-8) with a H₂O/MeOH gradient starting at 38:62 to 32:68 over 40 min followed by a maintained flow at 0:100 for 20 min, but none of the resulting fractions had any significant antiplasmodial activity. Fractions H3-4 (t_R 26.49 min) and H3-6 (t_R 35.18 min) were determined to be compounds **2** (0.3 mg) and **3** (0.4 mg), respectively. HPLC fraction H4 (3.9 mg) was highly active (IC₅₀ value <<< 1.25 µg/mL) and was separated into ten sub-fractions (H4-1 – H4-10) using a C₁₈ column with H₂O/MeOH starting at 35:65 to 26:74 over 10 min followed by 10 min of maintained flow at 26:74 then lastly a 5 min ramp to 0:100 followed by 10 min of maintained flow. Final purification was attempted on fraction H4-6 (0.6 mg; IC₅₀ value <<< 1.25 µg/mL; t_R 27.82 min) with diol HPLC using H₂O/MeCN starting at 60:40 to 59:41 over 8 min followed by a maintained flow at 0:100 for 8 min to afford 0.2 mg of **4** (t_R 7.12 min) with an estimated purity of 76% as judged from its ¹H NMR spectrum. Final purification was also attempted on fraction H4-8 (0.7 mg; IC₅₀ value

<<< 1.25 $\mu\text{g/mL}$; t_R 30.16 min) using the same method that produced **4** to afford 0.2 mg of **5** (t_R 8.00 min) with an estimated purity of 50% as judged from its ^1H NMR spectrum.

4.6.1 2-Heptylquinolin-4(1H)-one (1)—HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 509.3155 (calcd for $\text{C}_{32}\text{H}_{42}\text{N}_2\text{NaO}_2^+$ 509.3138), $[2\text{M}+\text{H}]^+ m/z$ 487.3339 (calcd for $\text{C}_{32}\text{H}_{42}\text{N}_2\text{O}_2^+$ 487.3319), $[\text{M}+\text{Na}]^+ m/z$ 266.1522 (calcd for $\text{C}_{16}\text{H}_{21}\text{NaO}^+$ 280.1515), $[\text{M}+\text{H}]^+ m/z$ 244.1705 (calcd for $\text{C}_{16}\text{H}_{22}\text{NO}^+$ 244.1696). ^1H NMR (500 MHz, CD_3OD) δ 8.21 (dd, J = 8.2, 1.5 Hz, 1H), 7.69 (ddd, J = 8.4, 7.0, 1.5 Hz, 1H), 7.58 (brd, J = 8.4 Hz, 1H), 7.40 (ddd, J = 8.2, 7.0, 1.0 Hz, 1H), 6.24 (s, 1H), 2.78 – 2.65 (m, 2H), 1.77 (p, J = 7.8 Hz, 2H), 1.49 – 1.24 (m, 8H), 0.90 (t, J = 6.9 Hz, 3H).

4.6.2. (6-(2-(Methylamino)phenyl)benzo[d][1,3]dioxol-5-yl) methanol (2)—HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 537.1984 (calcd for $\text{C}_{30}\text{H}_{30}\text{N}_2\text{NaO}_6^+$ 537.1996), $[\text{M}+\text{Na}]^+ m/z$ 280.0929 (calcd for $\text{C}_{15}\text{H}_{15}\text{NNaO}_3^+$ 280.0944), $[\text{M}+\text{H}]^+ m/z$ 258.1120 (calcd for $\text{C}_{15}\text{H}_{16}\text{NO}_3^+$ 258.1125), $[\text{M}-\text{H}_2\text{O}+\text{H}]^+ m/z$ 240.1014 (calcd for $\text{C}_{15}\text{H}_{14}\text{NO}_2^+$ 240.1019). ^1H NMR (500 MHz, CDCl_3) δ 7.31 (ddd, J = 8.7, 7.5, 1.6 Hz, 1H), 7.03 (s, 1H), 7.00 (dd, J = 7.5, 1.6, 1H), 6.85 – 6.82 (m, 1.0 Hz, 1H), 6.75 (brd, J = 8.1 Hz, 1H), 6.69 (s, 1H), 6.01 (s, 2H), 4.28 (d, J = 12.0 Hz, 1H), 4.21 (d, J = 12.0 Hz, 1H), 2.75 (s, 3H). ^1H NMR (500 MHz, CD_3OD) 7.22 (td, 7.8, 1.6 Hz, 1H), 7.06 (s, 1H), 6.90 (dd, 7.3, 1.7 Hz, 1H), 6.73 – 6.67 (m, 2H), 6.59 (s, 1H), 5.97 (d, 1.8 Hz, 2H), 4.25 (d, 12.8 Hz, 1H), 4.21 (d, 12.8 Hz, 1H), 2.72 (s, 3H).

4.6.3. 9-Hydroxy-10-methoxy-7H-pyrrolo[3,2,1-de]phenanthridin-7-one (3)—HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 553.1351 (calcd for $\text{C}_{32}\text{H}_{22}\text{N}_2\text{NaO}_6^+$ 553.1370), $[2\text{M}+\text{H}]^+ m/z$ 531.1533 (calcd for $\text{C}_{32}\text{H}_{23}\text{N}_2\text{O}_6^+$ 531.1551), $[\text{M}+\text{Na}]^+ m/z$ 288.0613 (calcd for $\text{C}_{16}\text{H}_{11}\text{NNaO}_3^+$ 288.0631), $[\text{M}+\text{H}]^+ m/z$ 266.0803 (calcd for $\text{C}_{16}\text{H}_{12}\text{NO}_3^+$ 266.0812). ^1H NMR (500 MHz, CDCl_3) δ 8.06 (d, J = 3.6 Hz, 1H), 8.04 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.79 (s, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 6.91 (d, J = 3.6 Hz, 1H), 4.10 (s, 3H). ^1H NMR (500 MHz, CD_3OD) 8.07 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 3.6 Hz, 1H), 7.95 (s, 1H), 7.78 (d, 7.7 Hz, 1H), 7.74 (s, 1H), 7.50 (t, J = 7.7 Hz, 1H), 6.98 (d, J = 3.6 Hz, 1H), 4.03 (s, 3H).

4.6.4. (Z)-2-(Non-3-en-1-yl)quinolin-4(1H)-one (4)—HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 561.3430 (calcd for $\text{C}_{36}\text{H}_{46}\text{N}_2\text{NaO}_2^+$ 561.3451), $[2\text{M}+\text{H}]^+ m/z$ 539.3638 (calcd for $\text{C}_{36}\text{H}_{47}\text{N}_2\text{O}_2^+$ 539.3632), $[\text{M}+\text{Na}]^+ m/z$ 292.1665 (calcd for $\text{C}_{18}\text{H}_{23}\text{NNaO}^+$ 292.1672), $[\text{M}+\text{H}]^+ m/z$ 270.1859 (calcd for $\text{C}_{18}\text{H}_{24}\text{NO}^+$ 270.1852). ^1H NMR (500 MHz, CD_3OD) δ 8.21 (brd, J = 7.2 Hz, 1H), 7.69 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.58 (brd, J = 8.3 Hz, 1H), 7.40 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 6.23 (s, 1H), 5.45 – 5.39 (m, 2H), 2.78 (t, J = 7.2 Hz, 2H), 0.80 – 0.76 (m, 3H).

4.6.5. 2-(6-Methylheptyl)quinolin-4(1H)-one (5) (Isolated natural product)—HRESIMS $[2\text{M}+\text{Na}]^+ m/z$ 537.3453 (calcd for $\text{C}_{34}\text{H}_{46}\text{N}_2\text{NaO}_2^+$ 537.3451), $[2\text{M}+\text{H}]^+ m/z$ 515.3650 (calcd for $\text{C}_{34}\text{H}_{47}\text{N}_2\text{NaO}_2^+$ 515.3632), $[\text{M}+\text{Na}]^+ m/z$ 280.1674 (calcd for $\text{C}_{17}\text{H}_{23}\text{NNaO}^+$ 280.1672), $[\text{M}+\text{H}]^+ m/z$ 258.1865 (calcd for $\text{C}_{17}\text{H}_{24}\text{NO}^+$ 258.1852). ^1H NMR (500 MHz, CD_3OD) δ 8.21 (brd, J = 8.7 Hz, 1H), 7.70 (m, 1H), 7.58 (brd, J = 8.3 Hz, 1H), 7.40 (brt, J = 7.3 Hz, 1H), 6.24 (s, 1H), 2.74 – 2.71 (m, 2H), 0.88 (d, J = 6.6 Hz, 3H).

4.7. Synthesis

4.7.1. Synthesis of alcohols 12 and 13—An oven dried 2-necked round bottom flask, vacuum arm adaptor, magnetic stir bar, and rubber septum were assembled hot and cooled under vacuum and purged with nitrogen. Freshly distilled THF (5 mL) was added and cooled to 0 °C and 1 M borane THF complex was added dropwise. Alkene was then added dropwise and the reaction was allowed to warm up to room temperature and stirred for 24 hours. The solution was hydrolyzed with 3 M NaOH and 50% hydrogen peroxide and stirred for an additional 3 hours. The reaction mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine then concentrated under reduced pressure. The resulting oil was chromatographed with silica gel using 80:20 hexane/EtOAc to afford the primary alcohols **12** and **13**. This procedure was adopted from Hajare et al.²⁷

4.7.2. 4-Methyl-1-hexanol (12)—The compound was obtained from 3.17 g (32.3 mmol) of **9** (4-methyl-1-hexene) and a large excess of 1 M borane THF solution (2 eq. or 65 mL or 65 mmol), and 12 mL of 3 M NaOH and 12 mL of 50% hydrogen peroxide were used for the hydrolysis. Silica gel column chromatography afforded 2.50 g of **12** (67% yield) as a clear liquid. GC-MS m/z 115.0. ¹H NMR (500 MHz, CDCl₃) 3.59 (t, J = 6.7 Hz, 2H), 1.90 (brs, 1H), 1.64 – 1.44 (m, 2H), 1.39 – 1.26 (m, 3H), 1.20 – 1.05 (m, 2H), 0.90 – 0.77 (m, 6H). ¹³C NMR (125 MHz, CDCl₃) 63.4, 34.4, 32.6, 30.4, 29.5, 19.2, 11.5. This compound has been previously reported.²⁸

4.7.3. 5-Methyl-1-hexanol (13)—Compound **13** was obtained from 2.8 g (28.2 mmol) of **10** (5-methyl-1-hexene) and 56.4 mL of 1 M borane THF solution (2 eq. or 56.4 mmol were used), and 11 mL of 3 M NaOH and 11 mL of 50% hydrogen peroxide were used for the hydrolysis. Silica gel column chromatography afforded 1.96 mg (60% yield) of product as a clear liquid. GC-MS m/z 115.1. ¹H NMR (500 MHz, CDCl₃) 3.62 (t, J = 6.7 Hz, 2H), 1.61 – 1.46 (m, 4H), 1.39 – 1.28 (m, 2H), 1.24 – 1.11 (m, 2H), 0.86, (d, J = 6.6 Hz, 6H). ¹³C NMR (125 MHz, CDCl₃) 63.2, 38.9, 33.2, 28.1, 23.7, 22.7 (double intensity). This compound has been previously reported.²⁹

4.7.4. General iodination procedure—An oven dried two-necked flask, vacuum arm adaptor, magnetic stir bar, and rubber septum were assembled warm and cooled under vacuum and purged with nitrogen. 1.3 eq of triphenylphosphine (PPh₃) and 1.4 eq of imidazole were added to the flask under constant N₂ flushing, sealed and pumped down and purged with N₂ three times. The solids were dissolved in freshly distilled diethyl ether and acetonitrile (2:1) and cooled to 0°C. The alcohols were added dropwise and stirred for 10 min. Under constant N₂ flushing 3 eq of iodine were added in portions, and stirred for another 2 hrs. The reaction mixture was diluted with 25 mL of *n*-pentane and washed with saturated Na₂S₂O₃ (3 × 25 mL), saturated CuSO₄ (3 × 25 mL), and lastly brine. The mixture was then concentrated under reduced pressure with minimal heat. The mixture was then chromatographed on silica gel with 100% *n*-pentane to afford the desired primary halides. This procedure was adopted from Tate et al.³⁰

4.7.5. 1-Iodo-3-methylhexane (14)—Iodide **14** was obtained from 664 mg (5.7 mmol) of dry **11** (3-methyl-1-hexanol), 1.95 g (7.4 mmol) PPh₃, 544 mg (8.0 mmol) imidazole, and

4.36 g (17.2 mmol) iodine to afford 797.9 mg (62%) of product. GC-MS m/z 226.1. ^1H NMR (500 MHz, CDCl_3) 3.28 – 3.13 (m, 2H), 1.86 (dddd, J = 13.8, 8.5, 7.2, 5.3 Hz, 1H), 1.70 – 1.49 (m, 2H), 1.42 – 1.07 (m, 4H), 0.91 – 0.85 (m, 6H). ^{13}C NMR (125 MHz, CDCl_3) 41.1, 38.6, 33.7, 20.0, 18.8, 14.4, 5.5. This compound has been previously reported.³¹

4.7.6. 1-Iodo-4-methylhexane (15)—Iodide **15** was obtained from 2.0 g (17.2 mmol) of dry **12** (4-methyl-1-hexanol), 5.87 g (22.4 mmol) PPh_3 , 1.64 g (24.1 mmol) imidazole, and 13.11 g (51.7 mmol) iodine to afford 2.59 g (67%) of product. GC-MS m/z 226.1. ^1H NMR (500 MHz, CDCl_3) 3.22 – 3.12 (m, 2H), 1.91 – 1.71 (m, 2H), 1.48 – 1.27 (m, 3H), 1.24 – 1.09 (m, 2H), 0.89 – 0.83 (m, 6H). ^{13}C NMR (125 MHz, CDCl_3) 37.5, 33.8, 31.4, 29.4, 19.3, 11.5, 7.7. This compound has been previously reported.³²

4.7.7. 1-Iodo-5-methylhexane (16)—The compound was obtained from 655.8 mg (5.7 mmol) of dry **13** (5-methyl-1-hexanol), with 1.9 g (7.4 mmol) PPh_3 , 540 mg (7.9 mmol) imidazole, and 4.3 g (16.9 mmol) iodine. Yield 809.2 mg (62%). GC-MS m/z 226.1. ^1H NMR (500 MHz, CDCl_3) 3.19 (t, J = 7.0 Hz, 2H), 1.80 (p, J = 7.2 Hz, 2H), 1.60 – 1.47 (m, 1H), 1.45 – 1.32 (m, 2H), 1.25 – 1.13 (m, 2H), 0.88 (d, J = 6.6 Hz, 6H). ^{13}C NMR (125 MHz, CDCl_3) 37.9, 33.9, 28.5, 28.0, 22.7 (double intensity), 7.5. This compound has been previously reported.³³

4.7.8. General ketoester procedure—An oven dried two-necked flask, vacuum arm adaptor, magnetic stir bar, and rubber septum were assembled hot and cooled under vacuum and purged with N_2 . The apparatus was charged with freshly distilled THF and 2.5 eq. of diisopropyl amine and cooled to -78°C . While stirring, 2.5 eq. of 2.5 M *n*-butyllithium were added dropwise. Once the addition was completed the mixture was warmed to 0°C and stirred for 30 min. The mixture was returned to -78°C and 1 eq. of ethyl acetoacetate were added dropwise and then the mixture was stirred for 1 hour. Lastly, 1.1 eq. of iodinated alkane were added dropwise and the mixture was allowed to reach room temperature while stirring for 18 hours. The reaction was quenched with 3 M HCl and extracted 3×50 mL with diethyl ether. The combined organics were dried over sodium sulfate and concentrated under reduced pressure. The crude yellow oil was then purified with silica gel chromatography 95:5 hexane/diethyl ether to afford the long chain ketoesters. This procedure was adopted from Lu et al.³⁴

4.7.9. Ethyl 7-methyl-3-oxodecanoate (19)—LDA (2 equiv.) was prepared from 4.46 mmol diisopropylamine and 1.8 mL (4.46 mmol) of 2.5 M *n*-butyllithium in hexanes. The LDA solution was treated with ethyl acetoacetate (2.23 mmol) to give dianion (**18**), which was treated with **14** (1.1 eq. or 2.48 mmol) for the alkylation. Column chromatography with 5:1 Hex/ Et_2O afforded 305 mg (61% yield) of product as a yellow oil. HRESIMS $[2\text{M}+\text{H}]^+$ m/z 457.3539 (calcd for $\text{C}_{26}\text{H}_{49}\text{O}_6^+$ 457.3524), $[\text{M}+\text{Na}]^+$ m/z 251.1618 (calcd for $\text{C}_{13}\text{H}_{24}\text{NaO}_3^+$ 251.1618). ^1H NMR (500 MHz, CDCl_3) 4.18 (q, J = 7.1 Hz, 2H), 3.41 (s, 2H), 2.50 (t, J = 7.4 Hz, 2H), 1.67 – 1.48 (m, 2H), 1.43 – 1.17 (m, 8H), 1.15 – 1.02 (m, 2H), 0.89 – 0.80 (m, 6H). ^{13}C NMR (125 MHz, CDCl_3) 203.2, 167.4, 61.4, 49.4, 43.5, 39.2, 36.4, 32.4, 21.1, 20.2, 19.6, 14.5, 14.2.

4.7.10. Ethyl 8-methyl-3-oxodecanoate (20)—LDA (2 equiv.) was prepared from 8.75 mmol diisopropylamine and 3.5 mL (8.75 mmol) of 2.5 M *n*-butyllithium in hexanes. The LDA was treated with 4.37 mmol of ethyl acetoacetate to give dianion **18**, to which was added 1.1 eq. (4.82 mmol) 1-iodo-4-methylhexane (**15**). Column chromatography with 5:1 Hex/Et₂O afforded 674 mg (67% yield) of product as a yellow oil. HRESIMS [M+Na]⁺ *m/z* 251.1611 (calcd for C₁₃H₂₄NaO₃⁺ 251.1618). ¹H NMR (500 MHz, CDCl₃) 4.17 (q, *J* = 7.1 Hz, 2H), 3.41 (s, 2H), 2.52 (t, *J* = 7.4 Hz, 2H), 1.62 – 1.49 (m, 2H), 1.36 – 1.18 (m, 8H), 1.14 – 1.03 (m, 2H), 0.87 – 0.77 (m, 6H). ¹³C NMR (125 MHz, CDCl₃) 203.1, 167.4, 61.4, 49.4, 43.2, 36.4, 34.3, 29.5, 26.6, 23.9, 19.2, 14.2, 11.5.

4.7.11. Ethyl 9-methyl-3-oxodecanoate (21)—LDA (2 equiv.) was prepared from 4.78 mmol diisopropylamine and 3.8 mL (4.78 mmol) of 2.5 M *n*-butyllithium in hexanes. The LDA was treated with 2.39 mmol of ethyl acetoacetate to give dianion **18**, and to this was added 2.65 mmol (1.1 eq.) of 1-iodo-5-methylhexane (**16**). Column chromatography with 5:1 Hex/Et₂O afforded 85.4 mg (16% yield) of product as a yellow oil. HRESIMS [2M+K]⁺ *m/z* 495.3093 (calcd for C₂₆H₄₈KO₆⁺ 495.3082), [M+Na]⁺ *m/z* 251.1620 (calcd for C₁₃H₂₄NaO₃⁺ 251.1618). ¹H NMR (500 MHz, CDCl₃) 4.19 (q, *J* = 7.2 Hz, 2H), 3.42 (s, 2H), 2.52 (t, *J* = 7.4 Hz, 2H), 1.59 (m, *J* = 7.4 Hz, 2H), 1.56 – 1.44 (m, 1H), 1.32 – 1.23 (m, 7H), 1.18 – 1.11 (m, 2H), 0.85 (d, *J* = 6.6 Hz, 6H). ¹³C NMR (125 MHz, CDCl₃) 203.2, 167.4, 61.5, 49.5, 43.2, 38.9, 29.4, 28.0, 27.2, 23.6, 22.7 (double intensity), 14.2.

4.7.12. Ethyl 3-oxoundecanoate (22)—LDA prepared from 8.76 mmol diisopropylamine and 3.5 mL (8.76 mmol) of 2.5 M *n*-butyllithium in hexanes was treated with 4.38 mmol of ethyl acetoacetate followed by 4.38 mmol (1.1 eq.) of 1-iodoheptane (**17**). Column chromatography with 5:1 Hex/Et₂O afforded 530 mg (53% yield) of product as a yellow oil. HRESIMS [2M+K]⁺ *m/z* 495.3060 (calcd for C₂₆H₄₈KO₆⁺ 495.3082), [2M+Na]⁺ *m/z* 479.3356 (calcd for C₂₆H₄₈NaO₆⁺ 479.3343), [M+Na]⁺ *m/z* 251.1623 (calcd for C₁₃H₂₄NaO₃⁺ 251.1618), [M+H]⁺ *m/z* 251.1800 (calcd for C₁₃H₂₅O₃⁺ 229.1798). ¹H NMR (500 MHz, CDCl₃) 4.11 (q, *J* = 7.2 Hz, 2H), 3.35 (s, 2H), 2.46 (t, *J* = 7.4 Hz, 2H), 1.51 (p, *J* = 7.4 Hz, 2H), 1.27 – 1.11 (m, 14H), 0.80 (d, *J* = 6.9 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃) 202.9, 167.2, 61.2, 49.2, 43.0, 31.8, 29.3, 29.1, 29.0, 23.4, 22.6, 14.1, 14.0. This compound has been previously reported.¹⁷

4.7.13. General β-enamino ester and 2-alkylquinolin-4(1H)-one procedure—An oven dried conical 5 mL vial and magnetic spin vane were cooled in a desiccator. Once cool the spin vane, a long chain ketoester, and aniline were added to the vial neat and stirred. A catalytic amount of InCl₃ was added and the vial was sealed with a septum, flushed with N₂, and stirred for 14 h. The reaction was then diluted with EtOAc and H₂O, and the aqueous layer extracted two more times with EtOAc. The organic layers were combined, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to give the crude β-enamino esters.¹⁵ The crude β-enamino esters were carried forward without purification to the next step. An oven dried two-necked round bottom flask, vacuum arm adaptor, magnetic stir bar, and rubber septum were assembled hot and cooled under vacuum. Once the apparatus had cooled to room temperature ~ 1 g of polyphosphoric acid (84% P₂O₅) was added under constant N₂ flushing. The apparatus was sealed and warmed to 110°C using a

sand bath under reduced pressure. Once stirring was possible, the system was purged with N₂, cooled to room temperature, and a spatula tip full of P₂O₅ was added and the process was repeated two more times. After the 3rd pump-purge the system was left at 110°C under N₂ and crude β-enamino ester was added via syringe. The mixture stirred for 24 h at 110°C, allowed to cool to rt, and ice cold water was added and stirred by hand with a glass rod. The mixture was transferred to a larger container and the pH was adjusted to 7 with solid sodium bicarbonate. The aqueous solution was then extracted with 3 × 25 mL of EtOAc, which was then dried over sodium sulfate and concentrated under reduced pressure.¹⁶

4.7.14. 2-(6-Methylheptyl)quinolin-4(1H)-one (5) (Synthetic Natural Product)—

The condensation reaction and cyclization were conducted with 3.01×10^{-4} mol of **21** and aniline. The crude product was then subjected to chromatography on a silica gel column with elution with 95:5 DCM/CH₃OH and the semi-pure product was then recrystallized from EtOAc to afford 13.6 mg (18% yield) of **5**. UV (MeOH) $\lambda_{\max}$ (log ϵ) 235 (5.23) nm; HRESIMS [2M+Na]⁺ m/z 537.3451 (calcd for C₃₄H₄₆N₂NaO₂⁺ 537.3451), [M+Na]⁺ m/z 280.1668 (calcd for C₁₇H₂₃NNaO⁺ 280.1672), [M+H]⁺ m/z 258.1849 (calcd for C₁₇H₂₄NO⁺ 258.1852). ¹H NMR (500 MHz, CD₃OD) δ 8.21 (dd, J = 8.2, 1.5 Hz, 1H), 7.68 (ddd, J = 8.5, 7.0, 1.5 Hz, 1H), 7.57 (dd, J = 8.5, 1.1 Hz, 1H), 7.38 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 6.22 (s, 1H), 2.75 – 2.69 (m, 2H), 1.76 (p, J = 7.5 Hz, 2H), 1.52 (dp, J = 13.3, 6.7 Hz, 1H) 1.47 – 1.42 (m, 4H), 1.24 – 1.15 (m, 2H), 0.87 (d, J = 6.7 Hz, 6H). ¹³C NMR (125 MHz, CD₃OD) δ 180.6, 157.2, 141.6, 133.4, 126.0, 125.5, 125.1, 119.1, 108.8, 40.0, 35.0, 30.5, 30.2, 29.1, 28.2, 23.0 (double intensity). ¹H NMR (500 MHz, CDCl₃) δ 11.02 (s, 1H), 8.36 (dd, J = 8.2, 1.5 Hz, 1H), 7.63 (dd, J = 8.4, 1.2 Hz, 1H), 7.58 (ddd, J = 8.4, 6.8, 1.5 Hz, 1H), 7.32 (ddd, J = 8.2, 6.8, 1.2 Hz, 1H), 6.22 (s, 1H), 2.73 – 2.56 (m, 2H), 1.78 – 1.61 (m, 4H), 1.44 (dp, J = 13.3, 6.6 Hz, 1H), 1.34 – 1.18 (m, 4H), 1.14 – 1.02 (m, 2H), 0.80 (d, J = 6.6 Hz, 6H). ¹³C NMR (125 MHz, CDCl₃) δ 179.1, 154.5, 140.4, 131.9, 125.7, 125.2, 123.7, 118.1, 108.6, 38.9, 34.6, 29.6, 29.1, 28.0, 27.2, 22.7 (double intensity).

4.7.15. 2-(4-Methylheptyl)quinolin-4(1H)-one (6)—

The condensation reaction and cyclization were conducted with 7.39×10^{-4} mol of **19**. and 7.39×10^{-4} mol of aniline. The crude product was then subjected to chromatography on a silica gel column with elution with 95:5 DCM/CH₃OH, and the semi-pure product was then recrystallized from EtOAc to afford 23.8 mg (13% yield) of **6**. HRESIMS [2M+Na]⁺ m/z 537.3444 (calcd for C₃₄H₄₆N₂NaO₂⁺ 537.3451), [2M+H]⁺ m/z 515.3617 (calcd for C₃₄H₄₇N₂NaO₂⁺ 515.3632), [M+Na]⁺ m/z 280.1668 (calcd for C₁₇H₂₃NNaO⁺ 280.1672), [M+H]⁺ m/z 258.1847 (calcd for C₁₇H₂₄NO⁺ 258.1852). ¹H NMR (500 MHz, CD₃OD) δ 8.21 (dd, J = 8.2, 1.5 Hz, 1H), 7.69 (ddd, J = 8.5, 7.0, 1.5 Hz, 1H), 7.58 (brd, J = 8.2 Hz, 1H), 7.39 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 6.23 (s, 1H), 2.76 – 2.64 (m, 2H), 1.88 – 1.67 (m, 2H), 1.52 – 1.37 (m, 2H), 1.35 – 1.19 (m, 4H), 1.17 – 1.05 (m, 1H), 0.93 – 0.84 (m, 6H). ¹³C NMR (125 MHz, CD₃OD) δ 180.6, 157.1, 141.6, 133.4, 126.0, 125.5, 125.1, 119.1, 108.9, 40.4, 37.5, 35.3, 33.5, 27.7, 21.1, 19.9, 14.7. ¹H NMR (500 MHz, CDCl₃) δ 11.56 (s, 1H) 8.36 (dd, J = 8.2, 1.5 Hz, 1H), 7.69 (ddd, J = 8.5, 1.1 Hz, 1H), 7.58 (ddd, J = 8.5, 7.0, 1.5 Hz, 1H), 7.32 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 6.23 (s, 1H), 2.72 – 2.61 (m, 2H), 1.81 – 1.62 (m, 2H), 1.39 – 1.07 (m, 6H), 1.05 – 0.94 (m, 1H), 0.80 (t, J = 7.1 Hz, 3H), 0.76 (d, J = 6.5 Hz, 3H). ¹³C NMR (125 MHz,

CDCl_3) δ 179.1, 154.9, 140.6, 131.9, 125.6, 125.2, 123.7, 118.4, 108.5, 39.3, 36.7, 34.9, 32.5, 26.7, 20.2, 19.6, 14.5.

4.7.16. 2-(5-Methylheptyl)quinolin-4(1H)-one (7)—The condensation reaction and cyclization were conducted with 1.32 mmol of **20** and aniline. The crude product was then subjected to chromatography on a silica gel column with elution with 95:5 DCM/ CH_3OH , and the semi-pure product was then recrystallized from EtOAc to afford 20.0 mg (6% yield) of **7**. UV (MeOH) λ_{max} (log ϵ) 235 (5.22) nm; HRESIMS $[\text{2M}+\text{Na}]^+$ m/z 537.3452 (calcd for $\text{C}_{34}\text{H}_{46}\text{N}_2\text{NaO}_2^+$ 537.3451), $[\text{2M}+\text{H}]^+$ m/z 515.3612 (calcd for $\text{C}_{34}\text{H}_{47}\text{N}_2\text{NaO}_2^+$ 515.3632), $[\text{M}+\text{Na}]^+$ m/z 280.1670 (calcd for $\text{C}_{17}\text{H}_{23}\text{NNaO}^+$ 280.1672), $[\text{M}+\text{H}]^+$ m/z 258.1853 (calcd for $\text{C}_{17}\text{H}_{24}\text{NO}^+$ 258.1852). ^1H NMR (500 MHz, CD_3OD) δ 8.20 (dd, J = 8.1, 1.5 Hz, 1H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.57 (dt, J = 8.4, 1.0 Hz, 1H), 7.38 (ddd, J = 8.1, 6.9, 1.0 Hz, 1H), 6.22 (s, 1H), 2.70 (t, J = 7.8 Hz, 2H), 1.73 (m, 2H), 1.36 (m, 5H), 1.15 (m, 2H), 0.86 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD) δ 180.6, 157.2, 141.6, 133.4, 126.0, 125.5, 125.1, 119.1, 108.8, 37.4, 35.6, 35.0, 30.5, 27.7, 19.5, 11.7. ^1H NMR (500 MHz, CDCl_3) δ 10.23 (s, 1H), 8.35 (dd, J = 8.2, 1.5 Hz, 1H), 7.58 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.53 (dd, J = 8.4, 1.2 Hz, 1H), 7.32 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 6.21 (s, 1H), 2.65 (t, J = 7.8 Hz, 2H), 1.71 (m, 2H), 1.27 (m, 5H), 1.07 (m, 2H), 0.80 (m, 6H). ^{13}C NMR (125 MHz, CDCl_3) δ 179.1, 154.0, 140.2, 132.0, 125.9, 125.2, 123.7, 117.8, 108.8, 36.4, 34.7, 34.4, 29.5, 29.2, 26.8, 19.3, 11.5.

4.7.17. 2-octylquinolin-4(1H)-one (8)—The condensation reaction and cyclization were conducted with 1.69 mmol of **22** and aniline. The crude product was then separated on a short C_{18} column with 75% aqueous methanol and the semi pure product was recrystallized from EtOAc to afford 91.3 mg (21% yield) of **8**. HRESIMS $[\text{2M}+\text{Na}]^+$ m/z 537.3453 (calcd for $\text{C}_{34}\text{H}_{46}\text{N}_2\text{NaO}_2^+$ 537.3451), $[\text{2M}+\text{H}]^+$ m/z 515.3642 (calcd for $\text{C}_{34}\text{H}_{47}\text{N}_2\text{NaO}_2^+$ 515.3632), $[\text{M}+\text{Na}]^+$ m/z 280.1677 (calcd for $\text{C}_{17}\text{H}_{23}\text{NNaO}^+$ 280.1672), $[\text{M}+\text{H}]^+$ m/z 258.1864 (calcd for $\text{C}_{17}\text{H}_{24}\text{NO}^+$ 258.1852). ^1H NMR (500 MHz, CD_3OD) δ 8.21 (brd, J = 8.1 Hz, 1H), 7.68 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.58 (brd, J = 8.4 Hz, 1H), 7.39 (brt, J = 7.5 Hz, 1H), 6.24 (s, 1H), 2.76 – 2.65 (m, 2H), 1.76 (p, J = 7.5 Hz, 2H), 1.49 – 1.20 (m, 10H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 180.5, 157.2, 141.6, 133.4, 125.9, 125.4, 125.1, 119.1, 108.8, 35.0, 33.0, 30.4, 30.3, 30.2, 30.2, 23.7, 14.4. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.47 (s, 1H), 8.02 (dd, J = 8.1, 1.4 Hz, 1H), 7.60 (ddd, J = 8.4, 6.8, 1.6 Hz, 1H), 7.52 (brd, J = 8.2 Hz, 1H), 7.26 (brt, J = 7.4 Hz, 1H), 5.91 (brd, J = 1.5 Hz, 1H), 2.57 (t, J = 7.6 Hz, 2H), 1.66 (p, J = 7.6 Hz, 2H), 1.27 (m, 10H), 0.84 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 177.0, 153.9, 140.2, 131.6, 124.8, 124.6, 122.9, 118.0, 107.7, 33.3, 31.3, 28.8, 28.7, 28.6, 28.5, 22.2, 14.0. This compound has been previously reported.³⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures 1 to 8. Compounds 1, 2, 3, 4, and 8 are known, and 5, 6, 7 and are new compounds.

Figure 2.

¹H NMR comparison of synthetic **5** with the small amount of the impure natural product **5** isolated from the plant in CD₃OD.

Scheme 1.
Synthesis of compounds **5** – **8**.

Table 1

Key ^1H and ^{13}C NMR Chemical Shifts from **5** – **8** in CD_3OD .

	5			6			7			8		
Posn	δ_{H} , mult. (J Hz) ^a	δ_{C}^b	δ_{H} , mult. (J Hz) ^a	δ_{C}^b	δ_{H} , mult. (J Hz) ^a	δ_{C}^b	δ_{H} , mult. (J Hz) ^a	δ_{C}^b	δ_{H} , mult. (J Hz) ^a	δ_{C}^b		
3	6.23, s	108.8	6.23, s	108.8	6.24, s	108.8	6.24, s	108.8	6.24, s	108.8		
5	8.21, dd (8.2, 1.5)	126.0	8.21, dd (8.2, 1.5)	126.0	8.21, dd (8.2, 1.5)	126.0	8.21, dd (8.2, 1.5)	126.0	8.21, brd (8.1)	125.1		
6	7.39, ddd (8.2, 6.9, 0.9)	125.1	7.39, ddd (8.2, 7.0, 1.1)	125.1	7.40, ddd (8.2, 6.9, 1.0)	125.1	7.39, brt (8.1)	125.1	7.39, brt (8.1)	125.9		
7	7.69, ddd (8.5, 6.9, 1.5)	133.4	7.69, ddd (8.4, 7.0, 1.5)	133.4	7.69, ddd (8.4, 6.9, 1.5)	133.4	7.68, ddd (8.4, 6.9, 1.4)	133.4	7.68, ddd (8.4, 6.9, 1.4)	133.4		
8	7.58, br d (8.5)	119.1	7.58, br d (8.4)	119.1	7.58, br d (8.4)	119.1	7.58, br d (8.4)	119.1	7.58, br d (8.4)	119.1		
1'	2.75 – 2.69, m	35.0	2.76 – 2.64, m	35.3	2.73 t (7.8)	35.0	2.76 – 2.65, m	33.0	2.76 – 2.65, m	33.0		
4'-Me	–	–	0.89, d (6.6)	19.9	–	–	–	–	–	–		
5'-Me	–	–	–	–	0.84, d (6.0)	19.5	–	–	–	–		
6'-Me	0.88, d (6.6)	23.0	–	–	–	–	–	–	–	–		
7'	0.88, d (6.6)	23.0	0.88, t (7.1)	14.7	0.86, t (7.2)	11.7	–	–	–	–		
8'	–	–	–	–	–	–	0.88, t (6.9)	14.4	–	14.4		

^aData (6) measured at 500 MHz; s = singlet, d = doublet, br d = broad doublet, t = triplet, brt = broad triplet, dd = doublet of doublets, ddd = doublet of doublets of doublets, and m = multiplet. J values are in Hz and are omitted if the signals overlapped as multiplets.

^bData (6) measured at 125 MHz.

Table 2Biological Data for **1 – 8**

Cmpd	<i>Plasmodium falciparum</i>		Activity against A2780 ovarian cancer cells (μ M)	Selectivity ratio
	Dd2 (nM)	3D7 (nM)		
1	1315 $\pm$ 292	1356 $\pm$ 329	35 $\pm$ 5	26.6
2	> 39000	–	> 78	NM
3	> 38000	–	> 75	NM
5	583 $\pm$ 330	501 $\pm$ 82	34 $\pm$ 5	57.7
6	330 $\pm$ 43	385 $\pm$ 78	29 $\pm$ 1	89.0
7	750 $\pm$ 93	921 $\pm$ 206	8.7 $\pm$ 2.1	11.6
8	3652 $\pm$ 470	3773 $\pm$ 575	12 $\pm$ 2	3.4

Artemisinin was used as a positive control for the Dd2 and 3D7 strains of *P. falciparum* with IC₅₀ values of 9.0 $\pm$ 2.7 nM and 5.9 $\pm$ 0.5 nM, respectively. Taxol was used as a positive control for the A2780 mammalian ovarian cancer cell line assay with an IC₅₀ value of 13.7 $\pm$ 0.7 nM.

NM = not meaningful