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ONE-COMPOUND-TWO-PHARMACOPHORES: DESIGN, SYNTHESIS AND ANTIMALARIAL ACTIVITIES OF CHLOROQUINOLINE-CASSIARIN

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ABSTRACT

*This paper describes the design and synthesis of a potential antimalarial compound, chloroquinoline-cassiarin (**6**) using 4,7-dichloroquinoline and the natural product barakol (**1**) extracted from the leaves of Cassia siamea as starting materials. In this paper a one compound-two-pharmacophores concept is introduced in which coupling of two known pharmacophores into one has been described. The concept is not only useful to organic synthesis but also to drug development researches. The synthesized compound **6** exhibited potent in vitro antimalarial activity at IC_{50} = 0.51 µM, and low toxicity where at 20 µM, 80% viability of HeLa cells was observed.*

Key Words: Chloroquinoline-cassiarin, Barakol, Antimalarial, Cassiarin, *Cassia siamea*

INTRODUCTION

Malaria, a curable and preventable disease, is still a life-threatening disease in Tanzania as well as in other endemic sub-Saharan African countries. However, according to WHO report, malaria cases have dropped globally from 262 million in 2000 to 214 million in 2015 which was 18% drop (WHO 2016).

Despite the efforts of eradicating malaria by using various therapies such as quinine, chloroquine, amodiaquine and many other known antiplasmodial compounds, the disease is still a number one killer in many African countries. The development of drug resistant strains of *Plasmodium* species has been known to thwart these (Ursos and Roepe 2002, Teixeira et al. 2011). Therefore, this calls for the development of compounds with not only antiplasmodial activity but also with needed pharmacokinetic activity. The plant *Cassia siamea* (Mjohoro or Mbaraka in Swahili, Tanzania) is known to be used for the treatment of periodic fever and malaria (Phaiphan et al. 2014, chanting et al. 2009, Oshimi et al. 2009, Kamagaté et al. 2014, Monton et al. 2015). Barakol (**1**) was the first natural product compound to be isolated from *C. siamea* (Hassanalli et al. 1969). Two other potent antimalarial alkaloids, cassiarins A and B have been isolated from this plant, thus in a way authenticating its folk medicinal use. The chemical structure of barakol (**1**) is similar to that of cassiarins in that the oxygen at position 4 is replaced by the nitrogen atom in the cassiarins. Unlike the cassiarins, barakol does not exhibit antimalarial activity. It is noteworthy that a number of cassiarins have been derived from barakol by replacing the O with N at the position 4 (Kanputhorn et al. 2010).

The cassiarins A and B are known to be less abundant (0.0008% and 0.0017% respectively) in their natural source than

compound **1** (0.3%) (Kanputhorn et al. 2010, Morita et al. 2007). Since an improved procedure for obtaining large quantities of compound **1** from *C. siamea* leaves has been established (Monton et al. 2015, Hassanalli et al. 1969, kanputhorn et al. 2010), availability and procurement of this material from its sources is cheap and guaranteed. Thus, it was envisioned that coupling of an amino-derivative of 4,7-dichloroquinoline (**5** with barakol (**1**) will result in a compound with higher antimalarial activities. The resulting coupled compound was hypothesized to have enhanced antimalarial activity and less prone to drug resistance, borrowing the ideas from the concept of combination therapy (WHO 2010, Nosten et al. 2000, Rosenthal 2003). In this approach, instead of combining two separate drugs, their biologically active parts are contained in one compound, a concept known as one-compound-two-pharmacophore.

Consequently, the study reported in this paper aims at utilizing barakol (**1**) which is a locally available natural product, to synthesize a new antimalarial compound (**6**) and evaluate its antimalarial activity.

EXPERIMENT

Plant Leaves and Material Collections

The leaves of *Cassia siamea* (Lam.) Irwin & Barneby (**Fig. 1**) were hand-picked from University of Dar es Salaam (UDSM) where the plants are grown for ornamental purposes. Identification and authentication was done at the herbarium in the Department of Botany, University of Dar es Salaam where a voucher specimen, FMM 3767 is deposited.



Figure 1: *Cassia siamea* at the UDSM campus (Photographed by T.M. Kalenga 2016)

Instruments and Materials (Chemicals and Reagents)

IR data recording was done using Alpha ATR FT-IR Spectrometer Bruker Optic GmH 2011, and Bruker A.G spectrometer (at either 300 MHz or 60 MHz NMR) was used for ^{1}H NMR and ^{13}C NMR spectra recording in deuterated methanol (MeOD) or DMSO. IR spectra for each compound were collected between 4000 and 500 cm^{-1} in appropriate resolution. Solvent removal from the products was done by using BUCHI Rotar Vapor R-205, vacuum controller V-800 heating bath B-490 and vacuum aspirator B-169.

Extraction and Isolation of Barakol (1)

A portion of the hand-picked leaves of *Cassia siamea* were sliced while fresh (600 g) and boiled for 45 minutes in 1.0L of 0.5% sulfuric acid, the procedure was repeated twice. Sodium carbonate (Na_2CO_3) was used to neutralize the extract, followed by extraction with DCM, then solvent evaporation to a quarter of the starting volume on a rotary evaporator under reduced pressure. A proportional amount of cold water (approx. 0˚C) was added, shaken and left to cool in a refrigerator for 1 hour; yellow needle-like solids of barakol (**1**) were filtered, washed with small amount of cold water, and dried in an air oven to obtain 1.8 g (0.3%).

The remaining portion of the leaves was air-dried, ground to powder (600 g) and the same procedure was repeated to afford 0.84 g (0.14%) of barakol (**1**): Mp 172-173 ºC (decomposition); the IR v_{max} cm^{-1} 3233.16 (OH), 1676.55 (C-C=C-O-C), 1561.01 and 1453.84 (aromatic ring). ^{1}H NMR (300 MHz, MeOH): δ_H 0.72 (s, 3H), 0.89 (s, 3H), 1.73 (1H), 4.71 (1H), 4.86 (1H) and 4.95 (1H). ^{13}C NMR (300 MHz, MeOH): δ_C 19.07, 20.66, 100.47, 105.69, 105.95, 108.83, 113.86, 133.94, 156.69, 160.44, 163.88, 170.89 and 184. 20.

Synthesis of Anhydrobarakol Chloride (3)

To a solution of **1** (0.500 g, 2.15 mmol) in methanol (4.0 mL), concentrated hydrochloric acid (1.0 mL) were slowly added at room temperature while stirring, the mixture was left stirring for 30 mins. Yellow solids were formed, the mixture was then cooled in ice cold water where 10 mL of THF were added for precipitation, followed by filtering, washing with THF to afford yellow solids of **3** [0.42 g (78%)]. Mp 203-204 ºC (decomposition); IR v_{max} cm^{-1} 3438.99 to 3495.72 (OH), 2852.11 to 3028.59 (sp^2 C-H), 2541.47 to 2752.56 (sp^3 C-H), 1659.92 (C-C=C-O-C), 1561.93 and 1534.71 (aromatic ring) and 853.04 (C-Cl) stretch.

Synthesis of *N*-(2-aminoethyl)-7-chloroquinolin-4-amine (5)

Ethane diamine (5.0 mL, 74.88 mmol) was added to dichloroquinoline (**7**) (5.0 g, 22.25 mmol) and refluxed for six hours at 92 ˚C, while the reaction mixture is monitored by TLC. Upon completion, the mixture was cooled, 1M NaOH (10 mL) was added and then extracted with dichloromethane (300 × 3 mL). The organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and then evaporated under reduced pressure to obtain 2.1 g of compound **5** as pale yellow solids in the yield 41.8%. Mp 126 ºC, the IR v_{max} cm^{-1} 3354.62 (N-H), 3243.49 (-NH_2), 2893.12 (C-H) and 1577.39 C=C (aromatic ring). ^{1}H NMR (300 MHz, MeOD): δ_H 2.96 (*m*, 2H), 3.41 (*m*, 2H); δ_H 6.52 (*d*, 1H), 7.36 (*d*, 1H), 7.75 (*s*, 1H), 8.07 (*d*, 1H) and 8.33 (*d*, 1H).^{13}C NMR (300 MHz, MeOD): δ_C 40.87, 46.36, 99.68, 118.78, 124.27, 125.98, 127.60, 136.29, 149.65, 152.42 and 152.77.

Synthesis of Chloroquinoline Cassiarin

To a 0.44 g (1.755 mmol) of anhydrobarakol chloride (**3**) in 4.0 mL methanol, 0.48 g (2.165 mmol) of N-(2-aminoethyl)-7-chloroquinolin-4-amine (**5**) were added at room temperature, the mixture was stirred for 1 hour. 2.0 mL of pyridine were then added and stirring continued for 70 minutes where 1.0 mL of concentrated HCl were added, the mixture was then left stirring for 14 hours. Through TLC the reaction was monitored, upon completion, methanol was evaporated followed by addition of THF for precipitation. Addition of 6.0 mL of distilled water, cooling in ice cold water and addition of Na_2CO_3 solution to pH 7.8 gave yellow solids. Filtering, washing and drying of the solids afforded 0.38 g (52%) of compound **6**. Mp 276-278 ºC (decomposition); IR v_{max} cm^{-1} 3204.44 (N-H), 1640.39 (C=O, conjugated), 1580. (C=C, aromatic) and 1139.83 (C-O). ^{1}H NMR (300 MHz, MeOH) δ_H 1.38 (*s*, 3H) and 2.38 (*s*, 3H), 3.36 (*m*, 2H), 3.34 (*m*, 2H), 6.77 (1H), 6.88 (*m*, 1H), 6.91 (*m*, 1H), 7.19 (1H), 7.52 (*m*, 1H), 7.82 (1H), 8.26 (*m*, H), 8.32 (*m*, 1H) and 8.44 (*m*, 1H).

Brine Shrimp Assay

Sterile artificial sea water in which brine shrimp (*Artemia salina*) eggs were hatched was prepared by dissolving 3.8 g of artificial sea salt in 1.0 L distilled water to make 3.8g/L. Solutions of compounds **1** and **6** in varying concentrations (240, 120, 80, 40 and 24 µg/mL) were prepared separately in DMSO. The solutions were added into a vial and in each 10 active *nauplii* were added then the volume adjusted to 5 mL with artificial sea water. The test experiment was performed in duplicates for each

concentration. The vials containing larvae were incubated under light for 24 hrs and the numbers of dead larvae were counted, percentage was calculated as: (Test – Control)/(control) times 100. The cytotoxicity of barakol (**1**) and compound **6** was determined from LC_{50} value per dose. Cyclophosphamide was used control experiment (Bycroft et al. 1970, Padumanonda et al 2007).

HeLa cell line Assay

Culturing of HeLa cells was done in 5% CO_2 at 37 ^oC incubator in Dulbecco's modified eagle medium (DMEM) with 4.5 g/L glucose and 4 mM L-glutamine. Treatment with 10% foetal calf serum and antibiotics; such as streptomycin (0.1 mg/mL), penicillin (100 mg/mL) and amphotericin (0.25 µg/mL). These cells were cultured in 96 well microplates at 10^4 cells per well and left overnight. Compound **6** (20 µM) was incubated with the cells for additional 24 hrs, and emetine standard was used as a positive control. Cell viability in the wells was assessed by adding 20 µL solution of mixture of MTT and PBS (phosphate-buffer solution) in the culture medium for each well and incubated for additional four hours at constant conditions. Fluorescence readings (excitation 560 nm, emission 590 nm) obtained for the individual wells were converted to percentage (%) cell viability relative to the average readings obtained from untreated control wells (Natarajan *et al* 2008).

Antimalarial Assay

Chloroquine sensitive strain (3D7) of parasite (*Plasmodium falciparum*) were cultured in 25mM medium of hepes and 2 mM L-glutamine, and supplemented with 2-4% (v/v) human erythrocytes, 0.5% (w/v) Albumax II (Thermo Fisher Scientific), 0.05mg/mL and 0.65 mM. Common standard culturing medium RPM1 1640 under 5% CO_2, 5% O_2 and 90% N_2 atmosphere cell culture were maintained at constant temperature (37 ^oC). For evaluation of antiplasmodial activity, parasite cultures (adjusted to 2% parasitemia, 1% haemotocrit) were placed in 96-well plates and incubated with 20 µM of compound **6** for 48 hours. Parasite lactate dehydrogenase (pLDH) enzyme was used to evaluate *in vitro* antiplasmodial activity. Nitro blue tetrazolium (NBT) is employed in the LDH determination, where NBT is reduced to formazan and detected at 620 nm. Duplicate wells for concentrations of both compound **6** and chloroquine standard were used. Enzyme (pLDH) activity in the individual wells was subsequently determined by removing 20 µL of the parasite cultures and mixing it with 125 µL colorimetric substrate solution containing 44 mM Tris buffer (pH 9), 0.18 M L-lactic acid, 0.13 mM acetylpyridine adenine dinucleotide, 0.39 mM NBT chloride, 0.048 mM phenazine ethosulfate and 0.16% (v/v) Triton X-100. Colour development was monitored by measuring absorbance at 620 nm in a Spectramax M3 plate reader (Molecular Devices). Absorbance values were converted to percentage (%) parasite viability relative to untreated control cultures. The mean IC_{50} inhibition at each concentration of compound **6** and standard were concurrently determined, and the parasite viability percentage value was calculated in the probit (Natarajan et al. 2008).

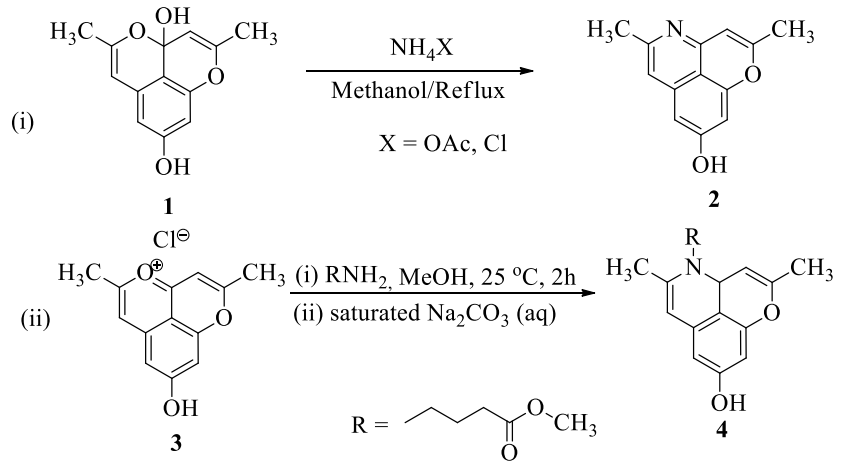
RESULTS AND DISCUSSION

Extraction and Purification of Barakol

Barakol (**1**) which is the starting material was successfully extracted from the leaves of *C. siamea* using the published procedure (Kanputhorn et al. 2010) and (Monton et al. 2015) which was adopted with slight modification. The obtained barakol (**1**) was characterized using FTIR, ^1H and ^{13}C NMR. The obtained data were consistent with those reported in the literature (Monton et al. 2015, Pavia et al. 2001).

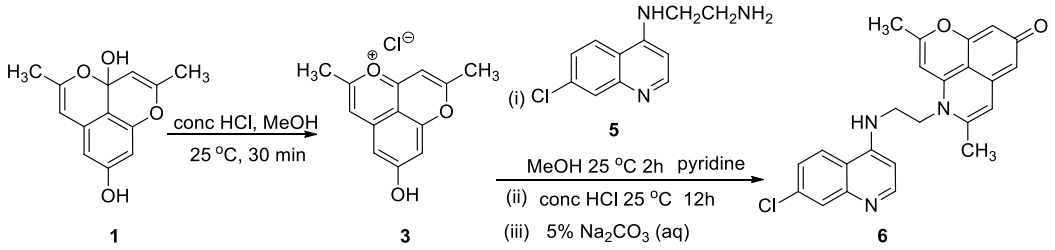
Synthesis

As illustrated in **Scheme 1**, the conversion of compound **1** to cassiarins can be achieved either by direct amination of compound **1** using ammonium salts (Scheme **1i**) or by amination of the chloride salt (**3**) of **1** (Scheme **1ii**). The approach of Scheme **1**(**ii**) is general and allows a one-step introduction of many amines unlike Scheme **1**(**i**) where only ammonia/its salts are used. The latter approach, with slight modification, was employed in the synthesis of the target compound (**6**) (Kanputhorn *et al* 2010).



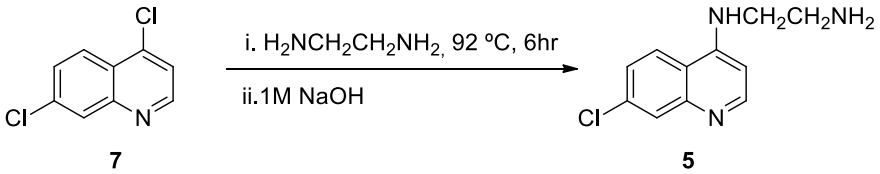
Scheme 1: Conversion of barakol **1** to Cassiarins

The first step towards the synthesis of the target molecule (**6**) involved the transformation of barakol (**1**) into precursor **3** using literature procedure (Kanputhorn *et al* 2010) and (Bycroft *et al*. (1970). Then, the obtained anhydrobarakol (**3**) was treated with compound **5** (*N*-(2-aminoethyl)-7-chloroquinolin-4-amine) in the presence of the pyridine in methanol to afford pale yellow crystals of the desired compound **6** as shown in **Scheme 2**.



Scheme 2: Conversion of **1** to **6**

However, the preparation of compound **5** which is one of the starting material precursor of this molecule was achieved by amination of dichloroquinoline (**7**) following the procedure described in the literature (Natarajan et al. 2008) (See **Scheme 3**). FT-IR, ^{1}H and ^{13}C NMR, confirmed the structural configuration of this compound.



7 **5**

Scheme 3: Synthesis of Precursor 5 from 4,7-Dichloroquinoline (7)

The structural characterization of the target molecule **6** was established using FT-IR and ^{1}H NMR. The ^{1}H NMR spectrum of compound **6** had two methyl proton signals characteristic of a tricyclic ring indicated by singlet signals at δ_H 1.38 (*s*, 3H) and 2.38 (*s*, 3H). Ethylene protons were observed as multiplets at δ_H 3.36 (*m*, 2H) and 3.34 (*m*, 2H). On the other hand, the methylene protons of the tricyclic ring and chloroquinoline were observed as nine multiplet signals at δ_H 6.77 (1H), 6.88 (*m*, 1H), 6.91 (*m*, 1H), 7.19 (1H), 7.52 (*m*, 1H), 7.82 (1H), 8.26 (*m*, H), 8.32 (*m*, 1H) and 8.44 (*m*, 1H). [20-22]. The stated spectroscopic data are consistent with the structure of the synthesized compound 4-(2-(7-chloroquinolin-4-ylamino)ethyl-2,5-dimethylpyranol[2,3,4-*ij*]isoquinoline-8(4H)-one (**6**). Like compound **1**, compound **6** in methanol was observed to be very sensitive to light, since within 48 hours of exposure it changed from pale green to dark green. For stability, solutions of both compounds demanded protection from sunlight.

Biological activities

Upon Brine Shrimp testing, compound **1** showed cytotoxic activity at LC_{50} = 3097 µg/mL while that of target compound **6** was found to be 333 µg/mL. The cytotoxicity of compound **6** seems to be influenced by the coupled moieties, this is because, compound **1** showed very low cytotoxic activity.

To confirm further the cytotoxic activity of compounds **1** and **6**, HeLa cell lines were used with emetine as a standard. At 20 µM of compound **6**, 80% viability of HeLa cells was observed, indicating the compound to be less toxic. The viability percentage of emetine standard was only high at low concentrations (0.012346-0.000457 µM).

The parasite (*Plasmodium falciparum*) lactate dehydrogenase (pLDH) enzyme was used as an indicator of *in vitro* antiplasmodial activity of the compounds. The assessment was done following the procedure described by Naghibi et al. (2013) (Naghibi et al. 2013) against the chloroquine sensitive (3D7) strain of parasite. The *in vitro* antimalarial evaluation of compound **6** was found to be IC_{50} = 0.51 µM while that of chloroquine (standard) was IC_{50} = 0.0011 µM. Compound **6** (an analogue of cassiarin B) exhibited higher activity than that of cassiarin B (IC_{50} = 21.9 µM). Therefore, coupling of the quinoline pharmacophore with that of cassiarin B has enhanced the activity of the compound.

CONCLUSION

To conclude, the design, synthesis and antimalarial assay of a new compound with two antimalarial pharmacophores has been achieved. Barakol (**1**) from the leaves of *C. siamea* was coupled to chloroquinoline derivative **5** to form compound **6**. *In vitro* antimalarial activity of the synthesized compound **6** is reported here together with its cytotoxicity results. Indeed the synthesized compound was found to be more potent than the corresponding cassiarin B.

Based on the good results from this preliminary study, work is underway to assess the effect of the length of the carbon chain linking the two pharmacophores and

as well as the effects of placing the linker at C-8 instead of the C-4 position. Since cassiarin A with an unsubstituted N is more potent than cassiarin B and since chloroquinoline-barakol analogue of cassiarin B is more potent therefore, it is expected that chloroquinoline-barakol analogue of cassiarin A may similarly be more potent.

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SUPPLEMENTARY INFORMATION

Supplementary information concerning this research is available.

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