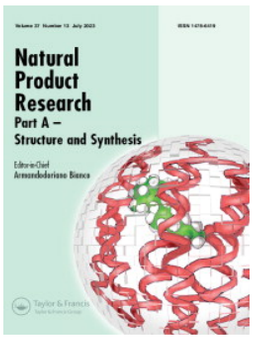


Taylor & Francis
Taylor & Francis Group

Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

A new C–C linked benzophenathridine–2-quinoline dimer, and the antiplasmodial activity of alkaloids from *Zanthoxylum holstzianum*

Denis Akampurira, Hoseah M. Akala, Solomon Derese, Matthias Heydenreich & Abiy Yenesew

To cite this article: Denis Akampurira, Hoseah M. Akala, Solomon Derese, Matthias Heydenreich & Abiy Yenesew (2023) A new C–C linked benzophenathridine–2-quinoline dimer, and the antiplasmodial activity of alkaloids from *Zanthoxylum holstzianum*, Natural Product Research, 37:13, 2161-2171, DOI: 10.1080/14786419.2022.2034810

To link to this article: https://doi.org/10.1080/14786419.2022.2034810

NATURAL PRODUCT RESEARCH
2023, VOL. 37, NO. 13, 2161–2171
https://doi.org/10.1080/14786419.2022.2034810

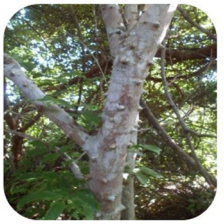
A new C–C linked benzophenathridine–2-quinoline dimer, and the antiplasmodial activity of alkaloids from *Zanthoxylum holstzianum*

Denis Akampurira[a,b], Hoseah M. Akala[c], Solomon Derese[a], Matthias Heydenreich[d] and Abiy Yenesew[a]

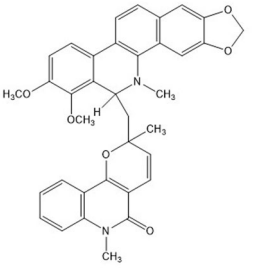
[a]Department of Chemistry, University of Nairobi, Nairobi, Kenya; [b]Department of Chemistry, Kyambogo University, Kyambogo, Kampala, Uganda; [c]Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRD-A/K), Kenya Medical Research Institute (KEMRI) - Walter Reed Project, Kisumu and Nairobi, Kenya; [d]Institut für Chemie, Universität Potsdam, Potsdam, Germany

ABSTRACT

The CH_2Cl_2/MeOH (1:1) extract of *Zanthoxylum holstzianum* stem bark showed good antiplasmodial activity (IC_{50} 2.5 ± 0.3 and 2.6 ± 0.3 μg/mL against the W2 and D6 strains of *Plasmodium falciparum,* respectively). From the extract five benzophenanthridine alkaloids [8-acetonyldihydrochelerythrine (**1**), nitidine (**2**), dihydrochelerythine (**3**), norchelerythrine (**5**), arnottianamide (**8**)]; a 2-quinolone alkaloid [*N*-methylflindersine (**4**)]; a lignan [4,4′-dihydroxy-3,3′-dimethoxylignan-9,9′-diyl diacetate (**7**)] and a dimer of a benzophenanthridine and 2-quinoline [holstzianoquinoline (**6**)] were isolated. The CH_2Cl_2/MeOH (1:1) extract of the root bark afforded **1**, **3**–**6**, **8**, chelerythridimerine (**9**) and 9-demethyloxychelerythrine (**10**). Holstzianoquinoline (**6**) is new, and is the second dimer linked by a C-C bond of a benzophenanthridine and a 2-quinoline reported thus far. The compounds were identified based on spectroscopic evidence. Amongst five compounds (**1-5**) tested against two strains of *P. falciparum*, nitidine (IC_{50} 0.11 ± 0.01 μg/mL against W2 and D6 strains**)** and norchelerythrine (IC_{50} value of 0.15 ± 0.01 μg/mL against D6 strain) were the most active.



Zanthoxylum holstzianum



ARTICLE HISTORY
Received 8 August 2021
Accepted 18 January 2022

KEYWORDS
Antiplasmodial; benzophenanthridine alkaloid; holstzianoquinoline; rutaceae; *Zanthoxylum holstzianum*

CONTACT Abiy Yenesew ayenesew@uonbi.ac.ke

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2022.2034810.

1. Introduction

The continuous emergence of resistance against antimalarial drugs means that there will always be need for new drugs to heal from malaria (Timothy and Wells 2011; Tajuddeen and Van Heerden 2019). In fact, if the recently reported resistance to artemisinin derivatives (Mbengue et al. 2015) escalates and spreads globally, the population at malaria risk area will be defenseless against this deadly parasite (Grimberg and Mehlotra 2011; Tse et al. 2019). In this regard, plants, especially those used in traditional medicinal practice, remain a potential source of new lead compounds. Amongst the different natural products known for their antiplasmodial activity over the years, the *Cinchona* alkaloids, particularly quinine from *C. officinalis* L. (as well as its synthetic derivatives), have demonstrated to be amongst the most important antimalarial products until apparition of parasitic resistance against them (Achan et al. 2011).

Some plants of the genus *Zanthoxylum* from Kenya and Uganda, for instance *Z. chalybeum,* are used to treat malaria (Bbosa et al. 2014; Waiganjo et al. 2020). These plants show antipyretic activity in either crude form or pure compounds (Jullian et al. 2006; Were et al. 2010). Besides the antiplasmodial activity attributed to the alkaloids of *Zanthoxylum* species, these plants show a wide range of biological activities including, antinociceptive, anticancer, anti-inflammatory, anti-oxidant and antimicrobial activities (Patino et al. 2012; Nooreen et al. 2019). Seven *Zanthoxylum* species are known to occur in Kenya (Beentje 1994). Investigation of some of these species have resulted in the isolation of phenanthridine alkaloids, alkamide derivatives, lignans, β-carboline alkaloids, triterpenes and sterols (Kato et al. 1996; Nyakobe et al. 2018; Kaigongi et al. 2020; Omosa et al. 2021a; Omosa et al. 2021b). Malaria being endemic to Africa, the importance of identifying a bioactive plant in the region cannot be over emphasized, since most people in malaria-endemic areas are likely to take herbal medicine before seeking treatment in the formal health facilities (Ocloo et al. 2014). On the basis of the African traditional medicine precedingly exposed, we have performed the quest of antiplasmodial activity of the stem bark and the root bark of *Zanthoxylum holstzianum* by means of isolation, structural characterization and antimalarial activity evaluation of the alkaloid content of the species.

2. Results and discussion

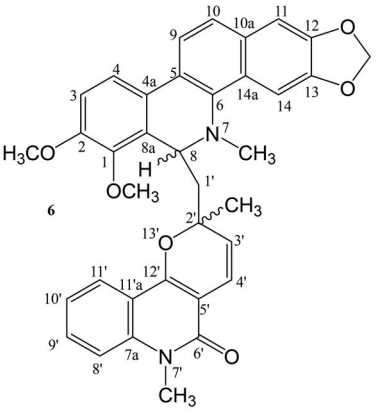
The stem bark of *Zanthoxylum holstzianum* was extracted with CH_2Cl_2/MeOH (1:1, vol/vol) by cold percolation. The extract was subjected to a combination of chromatographic separation yielding eight compounds. These include benzophenanthridine alkaloids, 8-acetonyldihydrochelerythrine (**1**) (Negi et al. 2011), nitidine (**2**) (Pei et al. 2011), dihydrochelerythrine (**3**) (Waterman et al. 1976), norchelerythrine (**5**) (Balawant et al. 1991), arnottianamide (**8**) (Hsiao and Chiang 1995); one symmetrical lignan [4,4′-dihydroxy-3,3′-dimethoxylignan-9,9′-diyldiacetate (**7**) (Martinez et al. 1999); a 2-quinoline alkaloid, *N*-methylflindersine (**4**) (Sheng et al. 1997); and a new dimer composed of the benzophenanthridine alkaloid dihydrochelerythrine and the 2-quinoline alkaloid *N*-methylflindersine, named holstzianoquinoline (**6**). This is the second dimer composed of benzophenanthridine and a 2-quinoline linked by C-C bond reported thus

far. In this study, the roots of this plant were also investigated, and led to the isolation of compounds **1**, **3**-**6**, **8**, chelerythridimerine (**9**) (Maclean et al., 1969), and 9-demethyloxychelerythrine (**10**) (Chen et al. 2005). The known compounds were identified by comparison of the spectroscopic data (Supporting Materials) with literature.

The new compound (**6** Figure 1) was isolated as colourless crystals with a green fluorescence on silica gel TLC plates under UV (366 nm) light. The compound gave a positive Dragendorff's reagent test. The HRMS showed a $[M]^+$ peak at *m/z* 588.2244 suggesting a molecular formula of $C_{36}H_{32}O_6N_2$, and indicating a dimeric alkaloid. In the EI-MS, the fragment ions at *m/z*, 348 ($[C_{21}H_{18}NO_4]^+$) and 226 ($[C_{14}H_{12}NO_2]^+$), suggest that compound **6** could be a dimer of dihydrochelerythrine (**3**) and *N*-methylflindersine (**4**), co-metabolites. The ^{1}H NMR (600 MHz, Figure S3) and ^{13}C NMR (150 MHz, Figure S4) in DMSO-d_6 showed duplication of signals, typical of diastereomeric mixtures. The ^{1}H NMR spectrum shows that one half of compound **6** is indeed dihydrochelerythrine (**3**). Thus, the presence of two sets of *ortho*-coupled protons [one set at δ_H 7.586/7.592 (*d*, $J = 8.7$ Hz, for H-4) and 7.034/7.049 (1H, *d*, $J = 8.8$ Hz, H-3), the second set at δ_H 7.788/7.893 (*d*, $J = 8.7$ Hz, H-9) and 7.516/7.524 (1H, *d*, $J = 8.5$ Hz, H-10)], two singlets aromatic protons (δ_H 7.230/7.301 for H-11, and δ_H 7.283/7.301 for H-14) are typical of benzophenanthridine alkaloids substituted with two methoxy, at C-1 (δ_H 3.545/3.707) and C-2 (δ_H 3.809/3.838), and methylenedioxy, at C-12 and C-13 [δ_H 5.962/6.053 and 6.136/6.174 (each *d*, $J = 1.1$ Hz)]. The benzylic proton H-8 being next to N-CH_3, (δ_H 2.459/2.584) appeared down field at δ_H 4.764/4.863, the multiplicity of which (*dd*, 10.1, 1.8 Hz)/(*dd*, $J = 10.5$, 2.3 Hz) suggest that it is coupled to a methylene protons of a substituent at C-8 (Section 3.5, Table S1) as in 8-acetonyldihydrochelerythrine (**1**) (Supporting Materials).

Further comparison of the NMR data (Table S1, Section 3.5) with *N*-methylflindersine (**4**) (Supporting Materials), showed that the substituent at C-8 is indeed *N*-methylflindersine (**4**), a co-metabolite. Thus, the ^{1}H NMR spectrum (Section 3.5, Table S1) revealed *cis*-olefinic protons [at δ_H 5.718/5.751 (H-3′) and 6.554/6.658 (*d*, $J = 10.0$ Hz, H-4′)], a three proton singlet for methyl group [at δ_H 1.407/1.583 (2′-CH_3)], and methylene protons [(CH_2-1′, at δ_H 1.670 (*dd*, $J = 15.0$, 2.2 Hz)/1.780 (*dd*, $J = 15.1$, 10.6 Hz), and 1.673 (*dd*, $J = 14.6$, 1.6 Hz)/1.803 (*dd*, $J = 14.7$, 10.1 Hz)], corresponds to a 2,2-dimethylpyrano moiety which is substituted at one of the two methyl groups (Wu and Chen 1993). In addition, a down field *N*-methyl signal at δ_H 3.600/3.638 which showed HMBC correlation with the amidic carbonyl (δ_C 159.64/159.71), four mutually coupled protons (Section 3.5, Table S1) of 1,2-disubstituted benzene, is consistent with one half of compound **6** being a 2-quinoline moiety with a modified 2,2-dimethylpyrano substituent. This half of the compound is connected to the other half (dihydrochelerythrine) through the 2,2-dimethylpyrano group where one of its methyl groups has become methylene due to its attachment to C-8 of dihydrochelerythrine moiety.

The C_8-$C_{1'}$ linkage between the two moieties was established from the HMBC spectrum (Figure S6), where the methyl protons at δ_H 1.407/1.583 (Me-2′) showed ^3J HMBC correlation with C-1′ (methylene carbon at δ_C 43.19/44.82), C-3′ (δ_C 126.49/127.28), and ^2J with C-2′ (δ_C 80.39/80.42). The connectivity between the two moieties was further supported by long-range HMBC correlation between H-8 and C-2′, and between the methylene protons CH_2-1′ and C-8 (Table S1). The H,H-COSY spectrum (Figure S5) showed a cross peak



Compound **6**

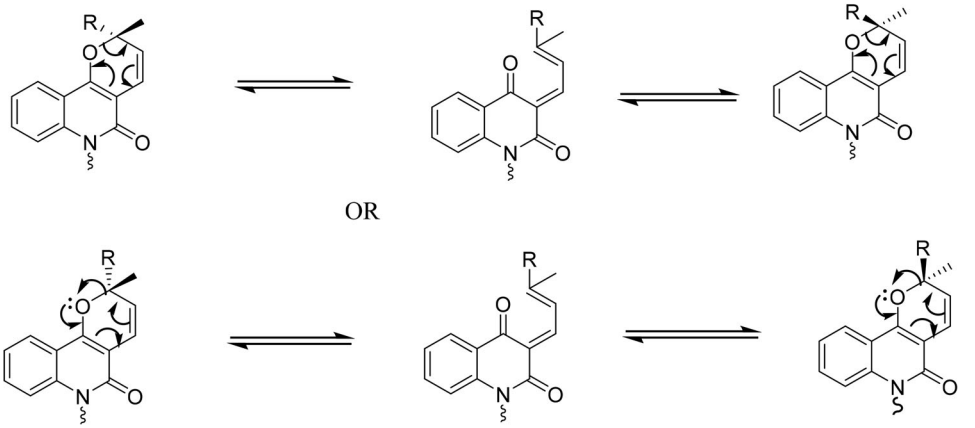


Figure 1. Structure, and proposed mechanism of epimerization of compound **6**.

between the benzylic proton (H-8) and the two methylene protons (CH_2-1′), supporting the C_8-C_1′ linkage. In agreement with this, the NOESY spectrum (Figure S8) showed correlation of H-8 (δ_H 4.764/4.863) with CH_2-1′ and CH_3-2′. The *N*-methyl group (δ_H 2.459/2.584) also showed HMBC correlation with C-8 and C-6 as in compound **1**. Complete assignment was done using HMBC (Figure S6) and HSQC (Figure S7) spectra, as well as comparing with the spectra data with those for compounds **1** and **4** (Supporting Materials). The data of the new compound (**6**), trivial name holstzianoquinoline, was further compared with that of simulanoquinoline (Wu and Chen 1993), the only related dimeric alkaloid isolated from *Zanthoxylum simulans,* and was in good agreement.

Interestingly, initial NMR analyses of compound **6** in in $CDCl_3$ gave one set of data for each atom (Table S1). However, when the analysis was done after removing the solvent from the NMR tube and dissolving it again in DMSO-d_6, doubled data set of nearly all ^{1}H and ^{13}C NMR signals was observed (Table S1, Section 3.5). Therefore, we suggest a racemization of the original compound two both possible diastereomers via a ring-opening followed by ring reclosure (Figure 1) has occurred as proposed by Harié et al. (1997).

The CH_2Cl_2/MeOH (1:1) extract of the stem bark of *Z. holstzianum* showed antiplasmodial activity with IC_{50} values of 2.5 ± 0.3 and 2.6 ± 0.3 μg/mL against W2 (chloroquine-resistant) and D6 (chloroquine-sensitive) strains of *Plasmodium falciparum*, respectively. Among the isolated compounds from this extract, nitidine (**2**) showed antiplasmodial activity, IC_{50} 0.11 ± 0.03 μg/mL and 0.11 ± 0.03 μg/mL; norchelerythrine (**5**), IC_{50} 7.3 ± 0.3 μg/mL and (0.15 ± 0.01 μg/mL); 8-acetonyldihydrochelerythrine (**1**), 4.0 ± 0.2 μg/mL and 3.8 ± 0.3 μg/mL; dihydrochelerythine (**3**), 3.8 ± 0.7 μg/mL and 3.2 ± 0.8 μg/mL while *N*-methylflindersine (4), 17.4 ± 2.0 μg/mL and 13.4 ± 0.9 μg/mL to both W2 and D6 strains, respectively. The results are summarized in Table S2.

According to Batista et al. (2009), a compound with $IC_{50} > 200$ μg/mL is considered to be inactive; IC_{50} 100-200 μg/mL, low activity; IC_{50} 20-100 μg/mL, moderate activity; IC_{50} 1-20 μg/mL, good activity; and $IC_{50} < 1$μg/mL, excellent or potent antiplasmodial activity. Among the isolated compounds, nitidine (against both W2 and D6 strains) and norchelerythrine (**5**) (against D6 strain) showed potent activity. 8-Acetonyldihydrochelerythrine (**1**), dihydrochelerythine (**3**), norchelerythrine (**5**) and *N*-methylflindersine (**4**) showed good activity (Table S2). The activity of nitidine against W2 and D6 and the activity of norchelerythrine (**5**) against D6 strain was higher than that of the crude extract, and similar to the activity of chloroquine, an antimalarial drug (Table S2). Gakunju et al. (1995), reported the activity of nitidine ranging from 0.009-0.11 μg/mL, which is in agreement with results obtained in this research. The relatively low activity of the 2-quinoline *N*-methylflindersine (**4**) compared to the benzophenanthridine alkaloids suggests that, the benzophenathridine nucleus appears to be important for the antiplasmodial activity of the extract.

3. Experimental

3.1. General

The NMR spectra were recorded using Varian-Mercury 200 MHz and/or Bruker Avance 500 and 600 MHz spectrometers. COSY, HSQC, NOESY and HMBC spectra were obtained using standard Bruker software. Chemical shifts were measured in ppm in δ values relative to the residue solvent signals. EI-MS were recorded at 70 eV on GC-TOF micromass spectrometer (micromass Wythenshawe, waters Inc. UK). ESIMS at 70 eV on a Micromass GC-TOF micromass spectrometer (Micromass, Wythenshawe, Waters, Inc. UK). Melting points were obtained on SMP 10 apparatus. Optical rotations were measured on a PerkinElmer 341-LC Polarimeter. The solvents used for chromatography were glass distilled.

3.2. Plant material

Zanthoxylum holstzianum (stem bark and root bark) was collected from Diani Veminant Forest, Coastal Province of Kenya in June 2012, and identified by Mr. Patrick Mutiso, a botanist from the Herbarium, Biology Department, University of Nairobi, where a specimen (voucher number, AD-001-2012) was deposited. Each of the plant material was air-dried and ground using a Willy mill.

3.3. Extraction and isolation of compounds from stem bark of Zanthoxylum holstzianum

The air dried and ground stem bark of *Zanthoxylum holstzianum* (3.4 kg) was defatted (4 L × 4) using *n*-hexane for 24 hours, in each case by cold percolation. This yielded 170 g of oily extract. The marc was then dried and further extracted with CH_2Cl_2/MeOH, 1:1 (4 L × 6) at room temperature, yielding 324 g (9.5%) of gummy extract. This extract was then acidified with 2 M HCl solution and extracted with dichloromethane followed by EtOAc, yielding 138 g of combined extract after concentration. The remaining aqueous layer was basified with 37% aqueous ammonia and extracted with CH_2Cl_2 followed by EtOAc two times, yielding 4 g of combined extract after concentration.

The basic organic extract (4 g), was adsorbed on silica gel (5 g) and loaded over silica gel (200 g). The column was eluted with *n*-hexane containing increasing amounts of CH_2Cl_2 (5%, 10%, 15%, 20%, 25%, 30%, 40%, 60%, 100%), followed by CH_2Cl_2 containing increasing amounts of methanol (2%, 4%, 10%, 20%, 50%). In total, 86 fractions, each *ca.* 250 mL were collected. Fractions that were eluted with 30% CH_2Cl_2 in *n*-hexane were combined and purified over Sephadex LH-20 (CH_2Cl_2/methanol; 1:1) yielding 8-acetonyldihydrochelerythrine (**1,** 11 mg). Fractions that were eluted with 20% methanol in CH_2Cl_2 yielded nitidine (**2,** 30 mg).

The acidic organic extract (138 g) was fractionated by VLC over preparative TLC grade silica gel, eluting with *n*-hexane, CH_2Cl_2, CH_2Cl_2/MeOH (1:1) and methanol yielding, 120 g, 32 g, 41 g and 3.1 g of fractions, respectively, after concentration. From the *n*-hexane fraction, colourless crystals of dihydrochelerythrine (**3,** 340 mg) were obtained. The CH_2Cl_2 fraction was subjected to column chromatography over silica gel (400 g) eluting with *n*-hexane containing increasing amounts of acetone (1%, 2%, 3%, 4%, 6%, 8%, 10%, 15%, 20%, 30%, 50%, 100%). The fractions eluted with 1% acetone in *n*-hexane yielded *N*-methylflindersine (**4,** 3 g). The fraction eluted with 2% acetone in *n*-hexane yielded norchelerythrine (**5,** 40 mg). Holstzianoquinoline (**6,** 62 mg) was obtained as white crystals from the fraction eluted with 6% acetone in *n*-hexane. The fraction eluted with 10% acetone in *n*-hexane was purified over Sephadex (eluting with CH_2Cl_2/MeOH, 1:1) and gave 4,4′-dihydroxy-3,3′ -dimethoxylignan-9,9′-diyl diacetate (**7,** 30 mg). Arnottianamide (**8,** 40 mg) was obtained from the fractions eluted with 15% acetone in *n*-hexane.

3.4. Extraction and isolation of compounds from root bark of Zanthoxylum holstzianum

The root bark of *Zanthoxylum holstzianum* (1.6 kg) was extracted as described in Section 3.3 yielding gummy extract (292 g). The extract was suspended in water and further extracted with EtOAc, yielding a 230 g extract that was fractionated by VLC as described in Section 3.3, further yielding *n*-hexane (120 g), CH_2Cl_2 (47 g), EtOAc (23 g), MeOH (15 g) fractions. The combined (70 g) CH_2Cl_2 and EtOAc fractions was subjected to column chromatography over silica gel (450 g) eluted with *n*-hexane containing increasing amounts of acetone (0.5%, 1%, 2%, 3%, 5%, 8%, 12%, 16%, 20%, 30%, 50%, and finally 100% acetone). The fraction eluted with *n*-hexane yielded

dihydrochelerythrine (**3,** 300 mg) after re-crystallization. The fraction eluted with 0.5% acetone in *n*-hexane gave *N*-methylflindersine (**4**, 4 g). The fraction eluted with 1% acetone in *n*-hexane gave white crystals of 8-acetonyldihydrochelerythrine (**1**, 13 mg) and norchelerythrine (**5**, 15 mg). Crystallization of the fraction eluted with 4% acetone in *n*-hexane afforded chelerythridimerine (**9**, 24 mg). The fraction eluted with 8% acetone in *n*-hexane gave holstzianoquinoline (**6**, 250 mg). The fraction eluted with 12% acetone in *n*-hexane gave arnottianamide (**8**, 20 mg). The fraction eluted with 15% acetone in *n*-hexane afforded 9-demethyloxychelerythrine (**10**, 300 mg) as white amorphous solid.

3.5. Physical and spectroscopic data of holstzianoquinoline (6)

Colourless crystals (from CH_2Cl_2), mp 210-214 °C; $[\alpha]_D^{24} = -1.56^\circ$ (*c* 0.41, CH_2Cl_2); R_f = 0.47 (20% ethyl acetate in *n*-hexane); UV (CH_2Cl_2, λ_{max}) : 230 (4.8), 247 (4.5), 260 (4.4), 280 (4.5), 290 (4.3) 332 (4.2), 349 (4.0) and 369 (3.7) nm; ^{1}H NMR (600 MHz, DMSO-d_6): δ_H 7.034 (d, J = 8.8 Hz, H-3), 7.049 (d, J = 8.8 Hz, H-3), 7.586/7.592 (d, J = 8.7/8.6 Hz, H-4), 7.592 (d, J=/8.6 Hz, H-4), 4.764 (dd, J = 10.1, 1.8 Hz, H-8), 4.863 (dd, J = 10.5, 2.3 Hz, H-8), 7.788 (d, J = 8.7 Hz, H-9), 7.793 (d, J = 8.7 Hz, H-9), 7.516* (d, J = 8.5 Hz, H-10), 7.524* (d, J = 8.5 Hz, H-10), 7.230 (s, H-11), 7.301 (s, H-11), 7.283 (s, H-14), 7.301 (s, H-14), 5.962 (d, J = 1.1 Hz, -OCH_2O-), 6.053 (d, J = 1.1 Hz, -OCH_2O-), 6.136 (d, J = 1.1 Hz, -OCH_2O-), 6.174 (d, J = 1.0 Hz, -OCH_2O-), 3.545 (s, 1-OCH_3), 3.707 (s, 1-OCH_3), 3.809 (s, 2-OCH_3, 3.838 (s, 2-OCH_3), 2.459 (s, 7-CH_3), 2.584 (s, 7-CH_3), 1.670 (dd, J = 15.0, 2.2 Hz, H-1′), 1.780 (dd, J = 15.1, 10.6 Hz, H-1′), 1.673 (dd, J = 14.7, 1.6 Hz, H-1′), 1.803 (dd, J = 14.7, 10.1 Hz, H-1′), 5.718 (d, J = 10.0 Hz, H-3′), 5.751 (d, J = 10.1 Hz, H-3′), 6.544, 6.658 (d, J = 10.0 Hz, H-4′), 7.519* (d, J = 8.4 Hz, H-8′), 7.547* (d, J = 8.4 Hz, H-8′), 7.624 (ddd, J = 8.6, 7.1, 1.5 Hz, H-9′), 7.658 (ddd, J = 8.5, 7.1, 1.5 Hz, H-9′), 7.208 (ddd, J = 8.1, 7.5, 1.0 Hz, H-10′), 7.329 (ddd, J = 8.0, 7.5, 0.9 Hz, H-10′), 7.969 (d, J = 7.9 Hz, H-11′), 7.971 (d, J = 8.0 Hz, H-11′), 1.407 (s, 3′-CH_3), 1.583 (s, 3′-CH_3), 3.600 (s, 7′-NCH_3), 3.638 (s, 7′-NCH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ_C 144.50/144.54 (C-1), 151.91/152.09 (C-2), 111.76/111.81 (C-3), 118.78/118.83 (C-4), 123.81/123.88 (C-4a), 130.60/130.75 (C-5), 139.05/139.07 (C-6), 53.00/53.02 (C-8), 128.66/128.84 (C-8a), 119.71/119.81 (C-9), 123.52/123.55 (C-10), 126.5 (C-10a), 104.12/104.35 (C-11), 146.99/147.14 (C-12), 147.52/147.70 (C-13), 100.07/100.16 (C-14), 128.66/128.84 (C-14a) 101.11/101.25 (-OCH_2O-), 60.10/60.16 (1-OCH_3), 55.61/55.66 (2-OCH_3), 42.24 (7-CH_3), 43.19/44.82 (br, C-1′), 80.39/80.42 (C-2′), 126.49/127.28 (C-3′), 115.26/116.74 (C-4′), 104.66/104.67 (C-5′), 159.64/159.71 (C-6′), 139.01 (C-7a′), 114.60/114.91 (C-8′), 131.10/131.25 (C-9′), 121.66/121.78 (C-10′), 122.36/122.41 (C-11′), 114.91, (C-11a′), 154.21/154.43 (C-12′), 26.76/27.91 (C-2′), 26.76/27.91 (2′-CH_3), 28.97/29.01 (7′-CH_3); EIMS *m/z*: 588 $[M]^+$, 589, 349, 348 ($[C21H18NO4]^+$), 333, 241, 226 ($[C14H12NO2]^+$); HRMS $[M]^+$ at *m/z* 588.2244 $C_{36}H_{32}O_6N_2$, Calculated for 588.2260).

3.6. Reference Plasmodium falciparum strains and reference antimalarial drugs

The following reagent was obtained through BEI Resources, NIAID, NIH: *Plasmodium falciparum*, Strain D6, MRA-285, and Strain W2, MRA-157 contributed by Dennis E. Kyle.

The reference antimalarials drugs chloroquine diphosphate (CQ) and Quinine (QN) were donated by the Worldwide Antimalarial Resistance Network External Quality Assurance Programme, Bangkok (Lourens et al. 2010).

3.7. Antiplasmodial assay

The crude stem bark extract and the pure compounds were assayed using a non-radioactive assay technique (Smilkstein et al. 2004), with modifications according to Johnson et al. (2007); Cheruiyot et al., 2016) to determine 50% growth inhibition of the cultured parasites. Two *Plasmodium falciparum* parasite strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) were maintained in continuous culture as described by Johnson et al. (2007), to attain a parasitemia of 3-8% based on counts infected versus uninfected red blood cells, confirming successful in vitro culture-adaptation. The crude extract, pure compounds and the reference drug were dissolved in 99.5% DMSO and diluted in complete Roswell Park Memorial Institute 1640 series of cell culture medium (RPMI 160) prepared as described by Akala et al. (2011). The basic culture medium was prepared from RPMI 1640 powder and the complete RPMI 1640 media was stored at 4 °C and used within 2 weeks.

Two-fold serial dilutions of chloroquine, the reference drug and test samples were prepared on a 96-well plate, making sure that the amount of DMSO is equal or less than 0.0875%. The culture-adapted *P. falciparum* at 3-8% parasitemia were first adjusted to 2% hematocrit and 1% parasitemia, then added onto the assay plate containing a range of drug doses and incubated in gas mixture (5% CO_2, 5% O_2 and 90% N_2) at 37 °C. The termination of the assay was done 72 hours later by addition of lysis buffer and incubating at room temperature in dark for 24 hours as described by Cheruiyot and coworkers (Cheruiyot et al., 2016). This duration allowed SYBR green I, a cyanide dye with reactive groups on nitrogen ends to get chemically linked to the *P. falciparum* nucleic acids resulting in a DNA-dye-complex that absorbs 485 nanometer blue light (λ_{max} = 497 nm) and emits 535 nanometer green light (λ_{max} = 520 nm). More interactions occurred in low drug-dose wells of the assay plate where more parasites replication was maximal during the 72 hours incubation giving more DNA than in higher dose wells. Per drug activity was quantified by analyses of dose response curves generated from the drug-dose dependent relative fluorescence units generated by the Genios tecan fluorescence reader from Baldwin Park, California, United States of America. The parasite growth quantified as mean ± standard deviation (Mean IC_{50} ± SD) as described by Johnson et al. (2007).

4. Conclusions

Overall, the CH_2Cl_2/MeOH (1:1) extracts of the stem bark and root bark of *Zanthoxylum holstzianum* afforded ten compounds including a new dimer, holstzianoquinoline (**6**). In antiplasmodial assay, nitidine (**2**) and norchelerythrine (**5**), were identified as the most active compounds.

Authors' contributions

A.D. carried out the isolation, structural determination and writing the manuscript; A.Y. structural determination and writing the manuscript; H.M.A. carried out antiplasmodial bioassay experiments; M.H., spectroscopic analysis and structural determination; S.D., research supervision and structural determination. All authors read the manuscript.

Acknowledgements

A.D. is grateful to the German Academic Exchange Services (DAAD) for a scholarship which was awarded through the Natural Products Research Network for Eastern and Central Africa (NAPRECA). The International Science Program (ISP Sweden, grant KEN-02), and the Kenya Medical Research Institute's Internal Research Grant L-183, are acknowledged for financial support. Mr. Patrick Mutiso for identification of the plant.

Disclosure statement

The authors declare no conflict of interest.

Funding

This research was partly supported by International Science Program (ISP Sweden, grant KEN-02), and the Kenya Medical Research Institute's Internal Research Grant L-183.

References

Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Rosenthal PJ, D'Alessandro U. 2011. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria . Malar J. 10:144.

Akala HM, Eyase FL, Cheruiyot AR, Omondi AA, Ogutu BR, Waters NC, Johnson JD, Polhemus ME, Schnabel DC, Walsh DS. 2011. Antimalarial drug sensitivity profile of western Kenya Plasmodium falciparum field isolates determined by a SYBR Green I in vitro assay and molecular analysis . Am J Trop Med Hyg. 85(1):34–41.

Balawant SJ, Kristi MM, Pelletier SW. 1991. Alkaloids of *Zanthoxylum budrunga* Wall. NMR assignments of Dihydrochelerythrine, (A)-Evodiamine and Zanthobungeanine. Phytochem Anal. 2: 20–25.

Batista R, Silva ADJ, de Oliveira AB. 2009. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules. 14(8):3037–3072.

Bbosa GS, Mwebaza N, Lubega A, Musisi N, Kyegombe DB, Ntale M. 2014. Antiplasmodial activity of leaf extracts of *Zanthoxylum chalybeum* Eng. BJPR. 4(6):705–713.

Beentje HJ. 1994. Kenya trees, shrubs and lianas: National Museum of Kenya, 372–374.

Chen JJ, Fang HY, Duh CY, Chen IS. 2005. New indolopyridinoquinazoline, benzophenanthridines and cytotoxic constituents form *Zanthoxylum integrifoliolum*. Planta Med. 71(5):470–475.

Cheruiyot AC, Auschwitz JM, Lee PJ, Yeda RA, Okello CO, Leed SE, Talwar M, Murthy T, Gaona HW, Hickman MR, et al. 2016. Assessment of the worldwide antimalarial resistance network standardized procedure for in vitro malaria drug sensitivity testing using SYBR green assay for field samples with various initial parasitemia levels. Antimicrob Agents Chemother. 60(4): 2417–2424. PMID: 26856829; PMCID: PMC4808143.

Gakunju DM, Mberu EK, Dossaji SF, Gray AI, Waigh RD, Waterman PG, Watkins WM. 1995. Potent antimalarial activity of the alkaloid nitidine, isolated from a Kenyan herbal remedy. Antimicrob Agents Chemother. 39(12):2606–2609.

Grimberg BT, Mehlotra RK. 2011. Expanding the antimalarial drug arsenal-now, but how? Review. Pharmaceuticals (Basel). 4(5):681–712.

Harié G, Samat A, Guglielmetti R, Van Parys I, Saeyens W, Keukeleire D, Lorenz K, Mannschreck A. 1997. Chiral 2-Aryl-2-methyl-2*H*-1-benzopyrans: synthesis, characterization of enantiomers, and barriers to thermal racemization. HCA. 80(4):1122–1132.

Hsiao J-J, Chiang H-C. 1995. Lignans from the wood of *Aralia bipinnate*. Phytochemistry. 39: 899–902.

Johnson JD, Dennull RA, Gerena L, Lopez-Sanchez M, Roncal NE, Waters NC. 2007. Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. Antimicrob Agents Chemother. 51(6):1926–1933.

Jullian V, Bourdy G, Georges S, Maurel S, Sauvain M. 2006. Validation of use of a traditional antimalarial remedy from French Guiana, *Zanthoxylum rhoifolium* Lam. J Ethnopharmacol. 106(3): 348–352.

Kaigongi MM, Lukhoba CW, Yaouba S, Makunga NP, Githiomi J, Yenesew A. 2020. In vitro antimicrobial and antiproliferative activities of root back extract and isolated constituents of *Zanthoxylum paracanthum* Kokwaro (Rutaceae). Plants. 9(7):920.

Kato A, Moriyasu M, Ichimaru M, Nishiyama Y, Juma FD, Nganga JN, Mathenge SG, Ogeto JO. 1996. Isolation of alkaloidal constituents of *Zanthoxylum usambalense* and *Zanthoxylum chalybeum* using Ion-pair HPLC. J Nat Prod. 59(3):316–318.

Lourens C, Watkins WM, Barnes KI, Sibley CH, Guerin PJ, White NJ, Lindegardh N. 2010. Implementation of a reference standard and proficiency testing programme by the World Wide Antimalarial Resistance Network (WWARN). Malar J. 9:375..

Martinez JC, Aldan J, Martha I, Cuca S, Luis E. 1999. Dibenzylbutane lignans from *Virola sebifera* leaves. Phytochemistry. 50:883–886.

Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, Rizk SS, Njimoh DL, Ryan Y, Chotivanich K, et al. 2015. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature. 520(7549):683–687.

Negi JS, Bisht VK, Bhandari AK, Shingh P, Sundriyal RC. 2011. Chemical constituents and biological activity of the genus *Zanthoxylum:* a review. Afr J Pure Appl Chem. 5:412–416.

Nooreen Z, Kumar A, Bawankule DU, Tandon S, Ali M, Xuan TD, Ahmad A. 2019. New chemical constituents from the fruits of *Zanthoxylum armatum* and its *in vitro* anti-inflammatory profile. Nat Prod Res. 33(5):665–672.

Nyakobe HO, Moraa M, Omosa LK, Mbaveng AT, Vanderament-Alexe N-N, Masila V, Okemwa E, Heydenreich M, Efferth T, Kuete V. 2018. Cytotoxicity of lupeol from the stem bark of *Zanthoxylum gilletii* against multi-factorial drug resistant cancer cell lines. Invest Med Chem Pharmacol. 1(1):10.

Ocloo A, Okpattah QW, Sakyiamah MM, Okine KN. 2014. Concurrent administration of aqueous extract of Cryptolepis sanguinolenta reduces the effectiveness of ART against *Plasmodium berghei* in rats. J. Appl. Pharm. Sci. 4:24–28.

Omosa LK, Mbogo GM, Korir E, Omole R, Seo E, Yenesew A, Heydenreich M, Midiwo JO, Efferth T. 2021a. Cytotoxicity of fagaramide derivative and cathin-6-one from *Zanthoxylum* (Rutaceae) species against multidrug resistant leukemia cells. Nat Prod Res. 35(4):579–586

Omosa LK, Nchiozem-Ngnitedem V-A, Mukavi J, Atieno Okoko B, Ombui Nyaboke H, Hashim I, Obegi Matundura J, Efferth T, Spiteller M. 2021b. Cytotoxic alkaloids from the roots of *Zanthoxylum paracanthum* (Mildbr) Kokwaro. Nat Prod Res.

Patino LOJ, Prieto RJA, Cuca SE. 2012. Zanthoxylum genus as potential source of bioactive compounds in phytomedicine. In: Biactive Compounds in Phytomedicine. Rasooli I, editor. InTech China. pp: 186–218. https://DOI:10.5772/26037.

Pei LV, Kanglun H, Longguan X, Xiaohua X. 2011. Palladium-catalyzed tandem reaction to construct Benzo[c]phenanthridine: application to the total synthesis of Benzo[c]phenanthridine alkaloids. Org Biomol Chem. 9(2011):3133–3135.

Sheng C, Inn T, Che T, Jih C, Ya C, Feng K, Matthias CL, Pezzuto JM. 1997. Pyranoquinoline alkaloids from *Zanthoxylum simulans*. Phytochemistry. 46(3):525–529.

Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. 2004. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrob Agents Chemother. 48(5):1803–1806.. PMID: 15105138.

Tajuddeen N, Van Heerden FR. 2019. Antiplasmodial natural products: an update. Malar J. 18(1): 404–462.

Tse EG, Korsik M, Todd MH. 2019. The past, present and future of anti-malarial medicines. Malar J. 18(1):93. https://doi.org/10.1186/s12936-019-2724-z.

Waiganjo B, Moriasi G, Onyancha J, Elias N, Muregi F. 2020. Antiplasmodial and cytotoxic activities of extracts of selected medicinal plants used to treat malaria in Embu County, Kenya. J Parasitol Res. 2020:8871375.

Waterman PG, Gray AI, Crichton EG. 1976. A comparative study of alkaloids of *Zanthoxylum leprieurii, Z. lemairei* and *Z, rubescense* from Ghana. Biochem Syst Ecol. 4(4):259–262.

Wells TNC. 2011. Natural products as starting points for future anti-malarial therapies: going back to our roots? Malar J. 10(1):1–2.

Were PS, Kinyanjui P, Gicheru MM, Mwangi E, Ozwara HS. 2010. Prophylactic and curative activities of extracts from *Warburgia ugandensis* Sprague (Canellaceae) and *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) against *Plasmodium knowlesi* and *Plasmodium berghei*. J Ethnophamacol. 130(1):158–162.

Wu S-J, Chen I-S. 1993. Alkaloids from *Zanthoxylum simulans*. Phytochemistry. 34:659–1661.