Antimalarial Activity of Cassane- and Norcassane-Type Diterpenes from *Caesalpinia crista* and Their Structure–Activity Relationship

Surya Kant KALUNI,∗a Suresh AWALE,∗a Yasuhiro TEZUKA,∗a Arjun Hari BANSKOTA,∗a Thein ZAW LINN,∗a Puji Budi Setia ASIH,b Din SYAFRUDDIN,b and Shigetoshi KADOTA∗,a

∗Institute of Natural Medicine, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan: and b Eijkman Institute for Molecular Biology; Jalan Diponegoro 69, Jakarta 10430, Indonesia.

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Malaria is one of the most life-threatening infectious diseases worldwide and claims the millions of peoples life each year. The appearance of drug-resistance *Plasmodium falciparum* has made the treatment of malaria increasingly problematic, and thus, it is a dire need to search the new alternatives of current drugs. In the present study, 44 cassane- and norcassane-type diterpenes isolated from *Caesalpinia crista* of Myanmar and Indonesia were evaluated for their antimalarial activity against the malaria parasite *Plasmodium falciparum* FCR-3/A2 clone in vitro. Most of the tested diterpenes displayed antimalarial activity, and norcaesalpinin E (28) showed the most potent activity with an IC₅₀ value of 0.090 µM, more potent than the clinically used drug chloroquine (IC₅₀ 0.29 µM). Based on the observed results, a structure–activity relationship has been established.

Key words cassane-type diterpene; antimalarial activity; structure–activity relationship; norcassane-type diterpene; *Caesalpinia crista*

Malaria remains the greatest human killer among parasitic infections, despite the continuous worldwide efforts to combat this disease and the attempts to eradicate the causative organisms.1) It is caused by the protozoan parasite of the genus *Plasmodium* and transmitted by mosquitoes of the genus *Anopheles*. Four species of *Plasmodium*, *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, can produce malaria in human. Among the four species, *P. falciparum* is the most widespread and dangerous. Malaria is an endemic disease in many parts of Asia, Africa, Central and South America, the Australian Continents, and some Caribbean islands. World Health Organization (WHO) estimates that each year 200—300 million people suffer from the case of malaria, resulting in up to 2.7 million deaths.2) The appearance of drug-resistance *P. falciparum* since 1960 has made the treatment of malaria increasingly problematic, and apparently the battle has not been successful.3) Since the historical discovery of quinine from *Cinchona* tree and the recent discovery of artemisinin from *Artemisia annua* L. (Asteraceae),4) there is anticipation that new leads may emerge from the tropical plant sources.

*Caesalpinia crista* Linn. (Fabaceae) is a famous medicinal plant widely distributed in tropical and subtropical regions of Southeast Asia. This plant is locally known as “Ka-Lain” in Myanmar and its seeds are used as anthelmintic, anti-inflammatory, and antimalarial agent.5) In Indonesia, it is known as “Bagore” and the decoction of roots have been used as a tonic and for the treatment of rheumatism and backache, while its seed kernels have been used as antimalarial and anthelmintic.6) In our preliminary study on the isolation of antimalarial agents from natural sources, we observed that the CH₃Cl₂ extract of seed kernels of *C. crista* collected from Indonesia and Myanmar exhibited significant inhibition of the growth of the malaria parasite *P. falciparum* FCR-3/A2 clone in vitro with IC₅₀ values of 0.095 and 0.21 µg/ml, respectively. Furthermore, the same CH₃Cl₂ extract of seed kernels of Indonesian *C. crista* also displayed significant in vivo antimalarial activity against the growth of *P. berghi* in mice.7) Thus, we carried out the detailed phytochemical investigation of this plant and isolated 40 new cassane- and norcassane-type diterpenes together with 18 known diterpenes.8)−11) In this paper, we report a biological profile on the antimalarial activity of the isolated diterpenes and their structure–activity relationships.

MATERIALS AND METHODS

Diterpenes Cassane- and norcassane-type diterpenes (Chart 1) used in this study were isolated from the CH₃Cl₂ extract of *C. crista* collected from Myanmar10−12) and Indonesia.7−9) The purity of each diterpene was checked by TLC and 1H-NMR spectrum, which did not show the presence of any impurity. Caesalpinsins MA (1), ME—MJ (2—7), ML (8), and MO (9) and norcaesalpinsins MC (10) and MD (11) were new compounds isolated from *C. crista* of Myanmar. Caesalpinsins C—F (12—15), H—K (16—19), and M—P (20—23) and norcaesalpinsins A—F (24—29) were new compounds isolated from *C. crista* of Indonesia. On the other hand, caesalmins B (30), C (31), E (32), and G (33); caesaldekarins e (34); 2-acetoxycaesaldekarin e (35); 2-acetoxy-3-deacetoxycaesaldekarin e (36); 6-acetoxy-3-deacetoxycaesaldekarin e (37); 14(17)-dehydrocaesalin F (38); bonducellpins B (39) and C (40); 7-acetoxybonducellpin C (41); 1-deacetoxy-1-oxoacesalin C (42); δ-caesalin (43); and 1-deacetylracesalin C (44) were known compounds.

Materials The malaria parasite *P. falciparum* FCR-3/A2 clone was a gift from Dr. M. Suzuki, Gunma University, Japan. RPMI-1640 medium, hypoxanthine, gentamycine, chloroquine, and Giemsa stain were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.), while 2-[4-(2-Hydroxyethyl)-1-piperaziny1]ethanesulfonic acid (HEPES) buffer was from Gibco BRL Products (Grand Island, NY, U.S.A.). The AB human serum and human erythrocytes were donated by healthy volunteers.

Parasite Culture The malaria parasite *P. falciparum* FCR-3/A2 clone was routinely propagated according to pre-
Previously published procedure. Briefly, the malaria parasite cultures (erythrocytic stage) were propagated in tissue culture flask containing RPMI-1640 medium supplemented with gentamycin 25 μg/ml, hypoxanthine 50 μg/ml, 25 mM HEPEs buffer, 25 mM sodium bicarbonate, 10% AB/H11001 human serum, 5% hematocrit, and human erythrocytes and incubated at 37 °C under 5% CO2. In erythrocyte (red blood cells), the parasites will undergo schizogonic developmental stage from the merozoite stage to mature schizont, which contains 12—36 daughter merozoites that eventually invades new red blood cells, which requires 36—48 h to proceed. The parasite growth was monitored by making blood smear, stained with Giemsa after 48 h to see the percentage of the red blood cells that were infected by the parasite.

Determination of the Antimalarial Activity

Anti-malarial activity of the isolated compounds was determined by the previously described procedure by Budimulja et al.14 In brief, each compound was separately dissolved in DMSO to obtain 10⁻³ M stock solution and kept at −20 °C until used. The different concentration of the compounds was freshly made from the stock solution. The malarial parasite P falciparum FCR-3/A2 clone was added to the 24-well culture plate which already containing different concentration of the compounds in RPMI-medium (total volume 1 ml having 1% parasitemia and 5% hematocrit/well) and incubated for 72 h at 37°C under 5% CO2 with medium change at 48 h. The growth of parasite was monitored by making a blood smear fixed with methanol and stained with Giemsa stain and number of the infected red blood cells were counted under the microscope. Chloroquine was used as a positive control. The concentration response parasite growth data were analyzed by a linear regression function using the Sigma-plot 2000 computer program to determine the 50% inhibitory concentration (IC50). The IC50 value is defined as the concentration of compound producing 50% parasite growth inhibition relative to untreated control.

RESULTS AND DISCUSSION

In the present study, 44 cassane- and norcassane-type diterpenes from C. crista of Myanmar and Indonesia have been tested for their inhibitory activity of the growth of the malarial parasite Plasmodium falciparum FCR-3/A2 clone. Majority of them exhibited various potencies of activity in a concentration-dependent manner (Table 1). The activities of diterpenes 21 (IC50, 0.12 μM), 25 (IC50, 0.26 μM), 28 (IC50, 0.090 μM), 29 (IC50, 0.14 μM), 36 (IC50, 0.098 μM), 38 (IC50, 0.20 μM), 39 (IC50, 0.24 μM), and 40 (IC50, 0.12 μM) were more potent than well-known antimalarial drug, chloroquine (IC50, 0.29 μM), and norcaesalpinin E (28) and 2-acetoxy-3-deacetoxycaesaldekarin e (36) showed the most potent activity with an IC50 value of 0.090 and 0.098 μM, respectively.
The activities of these diterpenes were greatly dependent on the nature of the substitution, and careful evaluation of the IC_{50} values led to the correlation between structure and activity.

**Effect of Hydroxyl Group** The presence of a hydroxyl group at C-7 plays crucial role for strong antimalarial activity. The diterpenes having a hydroxyl group at C-7 showed stronger activity than those with an acetoxyl substituent at C-7 (29 > 11, 39 > 18, 40 > 41) or without substituent at C-7 (39 > 15). The strong activity of caesalpinin N (21; IC_{50} 0.12 μM) might be attributable to the presence of a hydroxyl group at C-7.

**Effect of Acetoxyl Group** Majority of diterpenes isolated from the seed kernels of *C. crista* possessed the acetoxyl group at either of C-1, C-2, C-3, C-6, or C-7. In general, the simple cassane-type diterpenes having an acetoxyl substituent at C-1 showed stronger activity than those with a hydroxyl group at C-1 (13 > 16, 30 > 33, 31 > 44). Similarly, the activity of diterpenes possessing an acetoxyl group at C-6 was stronger than those having a hydroxyl group at C-6 (4 > 20, 13 > 22, 31 > 43). The monoacetoxyl-substituted diterpene having an acetoxyl group at C-1 showed stronger activity than that without substituent at C-1 (19 > 6).

Among the diacetoxyl-substituted diterpenes, those having acetoxyl substituents at C-1 and C-3 were more potent than those at C-1 and C-2 (12 > 23, 25 > 24). However, in the diterpene having aromatic ring-C, activity of 1,2-diacetoxyl-substituted diterpene was stronger than that of 1,3-disubstituted one [36 (IC_{50} 0.098 μM)] > 34 (IC_{50} 4.0 μM)]. Furthermore, 1,7-diacetoxyl-substituted diterpene was more potent than 1,6-disubstituted one [41 (IC_{50} 0.60 μM)] > 14 (IC_{50} 6.5 μM)].

On the triacetoxyl-substituted furanocassane diterpenes, those having acetoxyl substituents at C-1, C-2, and C-3 were more potent than those at C-1, C-6, and C-7 (27 > 10, 38 > 31). In general, diacetoxyl-substituted diterpenes having a ketone group at C-1 were more potent than triacetoxyl-substituted ones (11 > 10, 18 > 4, 42 > 31).

**Effect of Substituents on Ring C** The diterpenes isolated from *C. crista* showed wide range of structural variation and substitution pattern in ring C. In general, 17-norcassane-type diterpenes were more potent than the corresponding diterpenes having an aldehyde, methoxycarbonyl, or methyl group at C-17 (28 > 21 = 40 > 19). Similarly, norcaesalpinin B (25) was more potent than the diterpenes possessing a methylene, methyl, or methoxy carbonyl group at C-17 (25 > 12 > 1 > 3).

**CONCLUSION**

In the present study, we have reported the *in vitro* antimalarial activity of 44 cassane- and norcassane-type diterpenes isolated from *C. crista* of Myanmar and Indonesia. Most of the diterpenes displayed significant inhibition of growth of *Plasmodium falciparum* FCR-3/A2 clone *in vitro*. Among the diterpenes tested, norcaesalpinin E (28), a C-17-norcassane-type diterpene with an acetoxyl group at C-1 and a hydroxyl group at C-7, showed the most potent activity (IC_{50} 0.090 μM) which is stronger than the well-known antimalarial drug, chloroquine (IC_{50} 0.29 μM). From the observed antimalarial activities, we concluded that the presence of an acetoxyl group at C-1 and a hydroxyl group at C-7 plays the most important role for the antimalarial activity.

The inhibitory activity exhibited by these diterpenes could support the traditional use of the seed kernels of *C. crista* as an antimalarial drug.

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