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Original article

Isolation and identification of β-hematin inhibitors from *Flacourtia indica* as promising antiplasmodial agents☆

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Dedicated to Prof. William Fenical (SIO, UCSD, USA) for his successful efforts in the discovery of anticancer lead compounds marizomib and plinibulin.

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ABSTRACT

An ethanolic extract (A001) of the leaves and twigs of *Flacourtia indica* (Burm.f.) Merr., was purified to give a new phenolic glycoside, 2-(2-benzoyl-β-D-glucopyranosyloxy)-7-(1α,2α,6α-trihydroxy-3-oxocyclohex-4-enoyl)-5-hydroxybenzyl alcohol (**1**) together with poliothrysoside (**2**), catechin-[5,6-*e*]-4β-(3,4-dihydroxyphenyl)dihydro-2(3*H*)-pyranone (**3**), 2-(6-benzoyl-β-D-glucopyranosyloxy)-7-(1α,2α,6α-trihydroxy-3-oxocyclohex-4-enoyl)-5-hydroxybenzyl alcohol (**4**), chrysoeriol-7-O-β-D-glucopyranoside (**5**), and mururin A (**6**). Compound **6** significantly inhibited the *in vitro* growth of both a chloroquine-sensitive (3D7) and a chloroquine-resistant (K1) strain of *Plasmodium falciparum*. It forms a complex with hematin and inhibits β-hematin formation, suggesting that this compound act on a heme polymerization target.

1. Introduction

Malaria is a devastating infectious disease, causing great suffering and loss of human life. The global incidence of malaria is around 120 million clinical cases annually, with some 300 million people infected and 1–2 million dying from the disease each year [1]. Out of the five malaria species infecting humans, *Plasmodium falciparum* is responsible for the majority of deaths [2,3].

Quinine, an alkaloid isolated from the bark of *Cinchona officinalis*, was the drug of choice for malaria caused by *P. falciparum* until the 1940's [4,5] when other more effective drugs replaced it that have less unpleasant side effects. Chloroquine, a synthetic analog of quinine has long been used in the control of acute uncomplicated malaria caused by parasite of genus *Plasmodium* as the first line treatment until recently. The effectiveness of chloroquine against *P. falciparum* has declined as resistant strains of the parasite evolved [6]. Another natural product, a sesquiterpene lactone artemisinin and its semi-synthetic derivatives remain the most effective remedy for malaria for more than 20 years; however, their use also seems to be limited by recent cases of emerging resistance [7,8].

Presently, artemisinin-based combination therapies (ACT) are the recommended first line of treatment for falciparum malaria in all countries with endemic disease [9,10]. However, with the growing incidents of drug resistance threatening the effectiveness of currently available malaria therapies makes their long term use doubtful [11]. The global fight to control malaria requires a multi-faceted approach, one of them is to develop and identify new

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scaffolds with different targets and mechanism of action that could sustain the dreaded falciparum. Hence, taking into account the pivotal role of plant-derived compounds in drug discovery and the development of malaria chemotherapy, the isolation of new bioactive compounds or leads from medicinal plants seems to be a promising approach.

Flacourtia indica (Burm.f.) Merr., belonging to the family Flacourtiaceae, is a small deciduous tree indigenous to the Indian Peninsula. It is commonly known as 'Bilangra' or 'Baichi' in Hindi and 'Madagascar plum' in English [12]. Traditionally, the fruits of *F. indica* are used for the treatment of jaundice and enlarged spleen, while its gum resin is administered in the treatment of cholera [13]. The stems and leaves are used in traditional medicine against malaria in Madagascar and the Comoro Islands [14]. Previous phytochemical work on *F. indica* had led to the isolation of phenolic glycosides [15–19], a butyrolactone lignan, sterols [20], and flavonoids [17]. In a recent study, poliothrysoside isolated from the ethyl acetate extract of the plant showed promising *in vitro* antiplasmodial activity against the chloroquine-resistant strain (W2) of *P. falciparum* [21]. This prompted us to further explore this plant for new antimalarial chemotypes.

As part of a drug discovery programme from Indian medicinal plants [22,23], a new phenolic glycoside (**1**), together with five known compounds (**2**–**6**) [24–29], were isolated from the leaves and twigs of *F. indica*. Compounds **3**, **5**, and **6** are reported for the first time from this natural source. In this paper, we report the isolation and structure elucidation of the new compound, along with the antiplasmodial evaluation of the isolated phytoconstituents.

2. Results and discussion

2.1. Chemistry

The 95% ethanolic extract (A001) of the leaves and twigs of *F. indica* was suspended in water and partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate soluble fraction (F003) was subjected to a series of chromatographic techniques, leading to the isolation of a new phenolic glycoside (**1**), together with five known compounds (**2**–**6**). The known compounds were identified as poliothrysoside (**2**), catechin-[5,6-*e*]-4β-(3,4-dihydroxyphenyl)dihydro-2(3*H*)-pyranone (**3**), 2-(6-benzoyl-β-D-glucopyranosyloxy)-7-(1α,2α,6α-trihydroxy-3-oxocyclohex-4-enoyl)-5-hydroxybenzyl alcohol (**4**), chrysoeriol-7-O-β-D-glucopyranoside (**5**), and mururin A (**6**) (Fig. 1), by comparison of their spectroscopic data (see Supplementary material) with the reported literature values.

Compound **1** was obtained as a colorless gum. Its molecular formula was deduced to be $C_{27}H_{28}O_{14}$ by HRMS, having an index of hydrogen deficiency of 14. The IR spectrum displayed absorption bands indicating hydroxy (3378 cm^{-1}), carbonyl (1708 cm^{-1}) and phenyl ring (1627 cm^{-1}) functionalities. The ^1H NMR spectrum exhibited peaks characteristic of benzoyl, gentisyl, and glucose moieties suggesting a partial structure similar to that of poliothrysoside. A set of peaks at δ_H 8.08 (2H, d, J = 7.2 Hz), 7.46 (2H, m) and 7.58 (1H, t, J = 6.7 Hz) represented a benzoyl group, and another set of peaks at δ_H 7.04 (1H, d, J = 8.8 Hz), 6.66 (1H, m) and 6.78 (1H, d, J = 2.7 Hz) represented a 1,2,5-trisubtituted gentisyl alcohol moiety. A doublet appearing at δ_H 5.15 (J = 6.2 Hz) corresponding to the anomeric proton was consistent with a β-oriented glucose. Acid hydrolysis of **1** afforded D-glucose ($[\alpha]_D$ +50.5) [30]. Two mutually coupled protons ($^1H-^1H$ COSY) appearing at δ_H 6.03 (1H, dd, J = 10.3, 2.5 Hz) and 6.70 (1H, dd, J = 10.3, 1.7 Hz) were designated to *cis* olefinic protons. Both the olefinic protons coupled with another proton at δ_H 4.89 (1H, m), indicative of an isolated allylic system (−CH=CH−CH).

The ^{13}C NMR spectrum, combined with the DEPT data, revealed the presence of two oxygenated methylene, 10 olefinic methine, seven oxymethine, two esterified carbonyl, one α,β-unsaturated carbonyl, one aliphatic quaternary, and four aromatic quaternary carbons, accounting for 27 carbon signals (Table 1). Fig. 2 shows selected COSY and HMBC correlations which permitted assignment of the relative positions of the atoms and the overall structure of compound. The resonances appearing at δ_C 85.9 (C-1‴), 77.2 (C-2‴), and 71.7 (C-6‴) represented three oxygenated carbons which showed HMBC correlations with the olefinic protons (H-4‴ and H-5‴). The olefinic protons showed correlation with the carbonyl carbon at δ_C 198.4 (C-3‴). Additional correlation between the proton at δ_H 4.42 (1H, s, H-2‴) with the ester carbonyl at δ_C 172.1 (C-7‴), indicated a 1, 2, 6-trihydroxycyclohexenoyl moiety linked to an ester functionality.

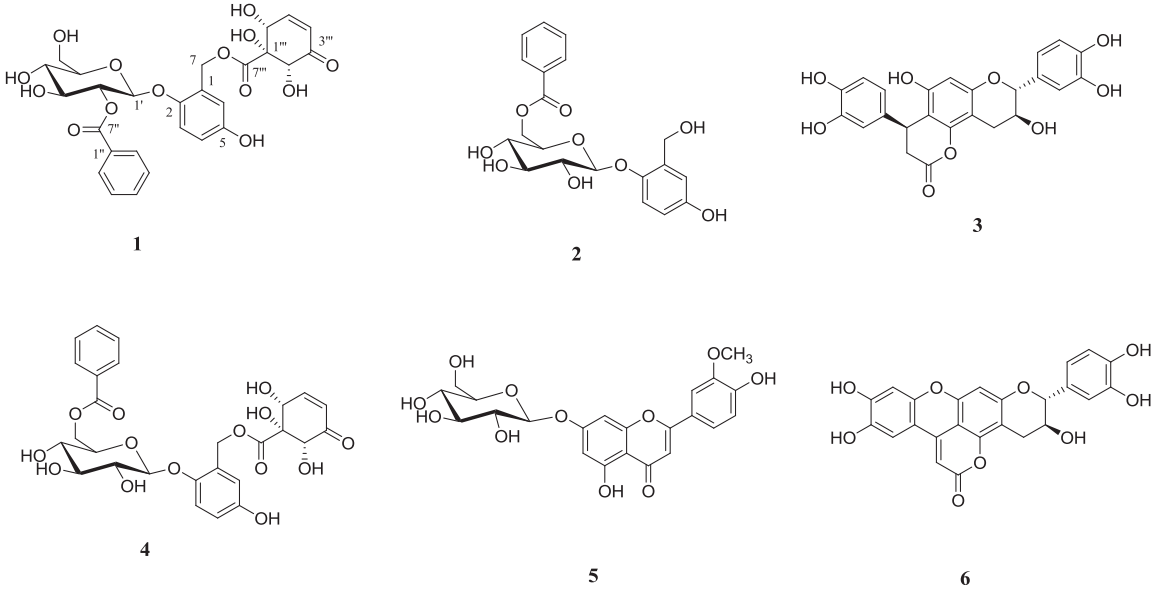

Fig. 1. Chemical structure of the isolated compounds from the leaves and twigs of *F. indica*.