Antiplasmodal and antileishmanial flavonoids from *Mundulea sericea*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Five known compounds (1–5) were isolated from the extract of *Mundulea sericea* leaves. Similar investigation of the roots of this plant afforded an additional three known compounds (6–8). The structures were elucidated using NMR spectroscopic and mass spectrometric analyses. The absolute configuration of 1 was established using ECD spectroscopy. In an antiplasmodial activity assay, compound 1 showed good activity with an IC50 of 2.0 μM against chloroquine-resistant W2, and 6.6 μM against the chloroquine-sensitive 3D7 strains of *Plasmodium falciparum*. Some of the compounds were also tested for antileishmanial activity. Dehydrolupinifolinol (2) and sericetin (5) were active against drug-sensitive *Leishmania donovani* (MHOM/IN/83/AG83) with IC50 values of 9.0 and 5.0 μM, respectively. In a cytotoxicity assay, lupinifolin (3) showed significant activity on BEAS-2B (IC50 4.9 μM) and HepG2 (IC50 10.8 μM) human cell lines. All the other compounds showed low cytotoxicity (IC50 > 30 μM) against human lung adenocarcinoma cells (A549), human liver cancer cells (HepG2), lung/bronchus cells (epithelial virus transformed) (BEAS-2B) and immortal human hepatocytes (LO2).

1. Introduction

Protozoan infections are responsible for serious human diseases, such as amoebiasis, Chagas’ disease, malaria, African sleeping sickness, leishmaniosis, and toxoplasmosis [1] that cause more than a million deaths annually [2]. The protozoan parasites that cause these diseases live in human blood or tissue, and are transmitted via blood probing insect vectors, mosquitoes or sand flies [3]. These diseases constitute the major health challenges for sub-Saharan countries including Kenya and Indian subcontinents [4–6]. In 2018, 228 million malaria cases occurred with 405,000 deaths worldwide. Leishmaniosis is a disease complex (visceral, cutaneous and mucocutaneous form) with estimated 0.7–1 million new cases annually. In 2018, more than 95% of new visceral leishmaniosis cases added from Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran, Iraq, Pakistan, the Syrian Arab Republic and Tunisia. Due to the development of resistance to current drugs [5,7,8], there is an urgent need to find alternative leads to fight malaria and leishmaniosis.

The plant genus *Mundulea* (family Leguminosae) is known for wide uses in traditional medicinal practices [9–12]. This family is a source of flavonoids and isoflavonoids, which have shown anticancer, [13,14] antimicrobial [15], antioxidant and antiparasitic activity [16,17] activities. We report the isolation and characterization of a new flavonoid along with seven known compounds from the leaves and roots of *Mundulea sericea*. The antiparasitic and antileishmanial activities, the cytotoxicity, and the induction of *in vitro* nitric oxide (NO) production in murine cells (indicator of antileishmanial activity), have been determined for selected compounds.

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2. Results and discussion

The air dried and powdered leaves of *Mundulea sericea* were extracted with CH₂Cl₂/MeOH (1:1). The extract was subjected to a combination of chromatographic separations that yielded five compounds (1–5, Fig. 1). These were identified using NMR spectroscopy and mass spectrometry as lupinofolin (1), [34] dehydrolupinofolin (2) [18] lupinofolin (3) [19], mundulinol (4) [13] and sericetin (5) [20] by comparison of their spectroscopic data with the corresponding data in the literature. Similar investigation of the roots of this plant led to the isolation of three known compounds, namely mutenone (6) [14], rotenone (7) [21] and striatine (8) [22].

Compounds 1–5 have fully substituted ring A, and in order to rule-out isomeric structures in this ring, detailed NMR analyses was conducted and this is illustrated by discussion on compound 1. It was obtained as a white amorphous solid, and its molecular formula, C₂₈H₂₀O₁₀, was determined by HREI-MS, which showed a [M + H]^⁺ peak at m/z 423.1807. The UV (λ<sub>max</sub> 260, 320 nm) and NMR spectral data (Table 1) are consistent with the presence of a flavanonol skeleton [23]. The ¹H NMR spectrum showed typical signals for ring C protons of a flavanonol skeleton at δ<sub>H</sub> 4.98 (H-2), 4.51 (H-3) and 3.60 (3-OH). In agreement with this, the ¹³C NMR spectrum showed signals at δ<sub>C</sub> 83.0 (C-2), 72.6 (C-3) and 196.2 (C-4). The nature of ring C was confirmed by the HMBC correlations with the corresponding data in the literature. Its relative configuration at C-2/C-3 was determined as trans from the large vicinal coupling constant (J = 11.9 Hz) between H-2 (δ<sub>H</sub> 4.98, d) and H-3 (δ<sub>H</sub> 4.51, d), suggesting a 1,2-diaxial relationship of these protons. Hence, two absolute configurations, (2R,3R) and (2S,3S), were possible [23]. The electronic circular dichroism (ECD) spectrum (Fig. 2) showed a negative Cotton effect within the range of the π → π⁺ transitions (ca. 300–340 nm), consistent with the (2R,3R) absolute configuration of 1 [23].

Flavonoids have previously been reported to be effective antimalarial and antileishmanial agents both in vitro [24,25], prompting the evaluation of the bioactivities of the crude extract and isolated compounds in this study. The crude extract of the roots of *Mundulea sericea* was tested for antimalarial activity against chloroquine-resistant (W2) and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum* using an established protocol (for details see Table 1).

### Table 1

| Position | δ<sub>C</sub> | δ<sub>H</sub> mult. (J in Hz) | HMBC (J<sub>2,3</sub>) |
|----------|----------------|-----------------------------|-------------------------|
| 2        | 83.0           | 4.98 d (11.9)               | C-3, C-4, C-1'          |
| 3        | 72.6           | 4.52 dd (11.9, 1.6)         | C-2, C-4, C-1'          |
| 4        | 196.2          |                            |                         |
| 4a       | 100.4          |                            |                         |
| 5        | 156.4          |                            |                         |
| 6        | 103.4          |                            |                         |
| 7        | 160.9          |                            |                         |
| 8        | 109.4          |                            |                         |
| 8a       | 159.5          |                            |                         |
| 1'       | 128.8          |                            |                         |
| 2'/6'    | 129.1          | 7.40 AA'                    | C-2, C-1', C-3'/5', C-4' |
| 3'/5'    | 115.6          | 6.84 XX'                    | C-1', C-2'/6', C-4'     |
| 4'       | 156.4          |                            |                         |
| 2''      | 78.6           |                            |                         |
| 3''      | 126.5          | 5.53 d (10.1)              | C-6, C-2''              |
| 4''      | 115.5          | 6.64 d (10.0)              | C-7, C-6, C-2''         |
| 2''-(CH₂)₂ | 28.5      | 1.45                        | C-2'', C-3', C-4''      |
| 1''      | 21.4           | 3.19 m                      | C-8, C-2'', C-3''       |
| 2''      | 122.2          | 5.12 m                      | C-1'', C-4'', C-5''     |
| 3''      | 131.5          |                            |                         |
| 4''-CH₃  | 17.9           | 1.60 br s                   | C-2'', C-3'', C-5''     |
| 5''-CH₃  | 26.0           | 1.64 br s                   | C-2'', C-3'', C-4''     |
| 5-OH     | 11.37 s        |                            | C-4a, C-5, C-6          |

**Fig. 1.** Structures of isolated compounds.

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[Raw text continues with detailed discussion and data analysis.]
Supplementary Information) [26]. The extract showed antiplasmodial activity with IC\textsubscript{50} values of 0.6 and 1.8 µg/mL against W2 and 3D7 strains, respectively. Some of the isolated compounds from this plant were also tested for antiplasmodial activity (Table 2). Compound 1 showed antiplasmodial activity with IC\textsubscript{50} value of 2.0 µM against the W2, and 6.6 µM against the 3D7 strains, while mundulolin (4) showed IC\textsubscript{50} of 5.9 µM against W2, and IC\textsubscript{50} of 2.4 against 3D7. Selected compounds were also evaluated for antileishmanial activity against L. donovani using an antimony-sensitive (MHOM/IN/83/AG83) and an antimony-resistant strains (MHOM/IN/89/GE1) (Table 2). Sericetin (S) showed antileishmanial activity against the antimony-sensitive (IC\textsubscript{50} 5.0 µM), and 38.0 µM against antimony-resistant (IC\textsubscript{50} 38.0 µM) strains. Dehydrolupinifolinol (2) was also active (IC\textsubscript{50} 9.0 µM) against the antimony-sensitive strain.

Nitric oxide (NO) is considered to be a crucial host anti-leishmanial defense substance. Compounds 2 and 5 showed visible increases in NO production in a cell culture with respect to a control in an amastigote assay. Compound 2 induced the highest NO production (3.3-fold) in the test cells, conferring a stronger NO-mediated protection.

The isolated compounds were also evaluated for their cytotoxicity against human lung adenocarcinoma (A549), human liver cancer (HePG2), human lung/bronchus cells (epithelial virus transformed, BEAS-2B) and immortal human hepatocytes (LO2) (Table 2). Dehydrolupinifolinol (2), isomundulolin (4) and sericetin (S) did not show significant toxicity against any of the cell lines (IC\textsubscript{50} > 100 µM). Lupinifolinol (1) was moderately cytotoxic to the normal cells BEAS-2B (IC\textsubscript{50} 36.6 µM) and LO2 (IC\textsubscript{50} 39.7 µM), while lupinifolin (3) was moderately cytotoxic to LO2 (IC\textsubscript{50} 36.6 µM) and strongly cytotoxic to BEAS-2B (IC\textsubscript{50} 4.9 µM) (Table 2).

### Table 2

| Sample | Antiplasmodial Activity (IC\textsubscript{50} µM) | Antileishmanial Activity (IC\textsubscript{50} µM) | Nitric Oxide Gene-ration | Cytotoxicity (IC\textsubscript{50} µM) |
|--------|---------------------------------|---------------------------------|-------------------|-------------------|
|        | W2 | 3D7 | MHOM/IN/83/AG83 | MHOM/IN/89/GE1 | RAW 264.7 | A549 | HePG2 | LO2 | BEAS-2B |
| 1      | 2.0 | 6.6 | >100 | >100 | NT | NT | 45.7 | 45.2 | 39.7 | 36.6 |
| 2      | NT | NT | 9.0 | >100 | 3.3 | 40.9 | >100 | >100 | >100 | >100 |
| 3      | 12.1 | 3.6 | >100 | >100 | NT | NT | 98.8 | 10.8 | 36.6 | 4.9 |
| 4      | 5.9 | 2.4 | >100 | >100 | 1.0 | 31.4 | >100 | >100 | >100 | >100 |
| 5      | NT | NT | 5.0 | 38.0 | NT | NT | NT | NT | NT | NT |
| CQ     | 0.08 | 0.008 | NT | NT | NT | NT | NT | NT | NT | NT |
| MF     | NT | NT | 5.5 | 6.7 | NT | NT | NT | NT | NT | NT |
| PT     | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |

MF: Miltefosine; PT: Paclitaxel; NT: Not Tested; CQ: chloroquine; W2: chloroquine-resistant strain of P. falciparum; 3D7: chloroquine-sensitive strain of P. falciparum; MHOM/IN/83/AG83: antimony-sensitive L. donovani; MHOM/IN/89/GE1: antimony-resistant L. donovani; RAW 264.7 = Abelson murine leukemia virus-induced tumor; A549 – human lung cancer cells; HePG2 – human liver cancer cells; LO2 – human hepatocytes normal cells; BEAS-2B – human hepatocytes normal cells.

Values indicate the number of fold change with respect to control.
white amorphous solids after further purification on a silica gel column (50 g), and eluted with n-hexane containing increasing amounts of CH₂Cl₂ (1 to 99% v/v). The fraction eluted with 3% EtOAc in n-hexane was washing with n-hexane and gave compound 4 (16 mg) as a yellow paste. The fraction eluted with 4% EtOAc in n-hexane afforded compound 5 (17 mg) as yellow crystals from CH₂Cl₂+n-hexane. The fraction eluted with 10% EtOAc in n-hexane yielded compound 6 (22 mg) as a white amorphous solid after further purification on a silica gel (50 g) column, eluting with n-hexane containing increasing amounts of CH₂Cl₂ (1 to 99% v/v). The fraction eluted with 75% EtOAc in n-hexane gave compound 2 (10 mg) as yellow amorphous solid after further purification on a Sephadex LH 20 column, eluting with CH₂Cl₂/MEOH (1:1) as the eluent.

3.3.2. Isolation of compounds from the roots of Mundulea sericea

Air dried roots of Mundulea sericea (965 g) were extracted (4 × 4 l) with CH₂Cl₂/MEOH (1:1) at room temperature and the solvent removed under reduced pressure. The crude extract (91.2 g), was adsorbed on silica gel, loaded onto silica gel (500 g) column, and eluted with n-hexane containing increasing amounts of EtOAc (1 to 99% v/v). The eluents were then pulled into 24 fractions. The fraction eluted with 6% EtOAc in n-hexane gave compound 3 (30 mg) as colourless crystals after further purification on a silica gel (50 g) column, eluting with n-hexane containing increasing amounts of CH₂Cl₂ (1 to 99% v/v). The fraction eluted with 6% EtOAc in n-hexane afforded compound 7 (17 mg) as white solids after further purification on Preparative TLC with n-hexane/EtOAc (7:3) as eluent. The fraction eluted with 6% EtOAc in n-hexane afforded compound 8 (50 mg) as yellow paste after further purification on centrifugal TLC, eluting with n-hexane/EtOAc (7:3). An equal volume of DMSO was added in control experiments. After 48 h incubation, MTT (5 mg/ml, 20 l per well) was added to each well and the plate was incubated for another 4 h at 37 °C. The reaction was then stopped with acidic isopropanol (0.4 ml 10 N HCl in 100 ml isopropanol, 100 l per well), and the absorbance was measured at 595 nm in a microplate reader (Bio-Rad, USA). The 50% inhibitory concentrations were determined from the plots of percent inhibition against increasing concentrations. Cytotoxic effect of the selected active compounds was also evaluated on RAW 264.7 cells in comparison to the reference drug Mitelofosine. Nitric Oxide generation from RAW 264.7 cells was assayed by using Griess reagent [31]. Briefly, cells supernatants were collected (60 h), Nitric Oxide generation was assayed by using Griess reagent [31], briefly, for the estimation of nitric oxide (NO) in RAW 264.7 cells, cells supernatants were collected and distributed (100 μl per well) in 96-well plates, and an equal volume of Griess reagent was added to each well, incubated for 15 min at 37 °C, and the absorbance was taken at 540 nm by an microplate reader (Bio-Rad, USA) [32]. Three or more independent experiments were performed in triplicate for each compound. Statistical analyses for all experiments were performed by one-way ANOVA followed by post hoc Holm-Sidak test with Sigma Plot software (version 11.0) [30].
Author Contributions

The authors contributed to this work as follows. Extraction and isolation of compounds was performed by C.C. and P.J.O. under the supervision of A.Y., A.N. and S.D.; NMR analyses was performed with the help of M.H. and M.E.; Spectroscopic characterization of the compounds was carried out by C.C., A.Y., M.H. and M.E.; antileishmanial tests and NO induction assay were performed by B.S. and A.H. under the supervision of C.P.; antiplasmodial tests were done by H.M.A.; cytotoxicity assays were performed by P.C. and L.J.Y. under the supervision of V.K.W.W. All authors contributed to the preparation of the manuscript.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

The following are available online at www.elsevier.com.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.5281/zenodo.3902746.

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