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Potent antitrypanosomal triterpenoid saponins from *Mussaenda luteola*

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ABSTRACT

Five new triterpenoid saponins, heinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (**1**), heinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**2**), 2α-hydroxyheinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (**3**), 2α-hydroxyheinsiagenin A 3-*O*-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**4**) and *N*-(2*S*, 3*R*, 4*R*-3-methyl-4-pentanolid-2-yl)-18-hydroxylanosta-8 (9), 22*E*, 24*E*-trien-27-amide-3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**5**) were isolated from the aerial parts of *Mussaenda luteola* Delile (Rubiaceae). Structural elucidation was based on the analysis of spectroscopic data (1D and 2D NMR) and HR-ESI-MS. Compound **1** showed potent antitrypanosomal activity with an IC_{50} value of 8.80 μM. Compounds **2**–**4** showed highly potent antitrypanosomal activity with IC_{50} values ranging between (2.57–2.84 μM) and IC_{90} values ranging between (3.36–4.35 μM), which are 5 fold greater than the positive control DFMO (IC_{50} and IC_{90} values of 13.06 and 28.99 μM, respectively). Compounds **1** and **2** showed moderate affinity to μ-opioid receptors with K_i values of 9.936 μM and 0.872 μM, respectively compared to a K_i value of 1.958 nM for the positive control, naloxone HCl.

1. Introduction

Mussaenda (Rubiaceae) is an important source of medicinal natural products. *Mussaenda* species are native to West Africa, the Indian subcontinent, South-East Asia, and Southern China [1]. There are more than 200 species of *Mussaenda* [1]. Some species have historically been used in Chinese and Fijian traditional medicine as a diuretic, antiphlogistic, antipyretic, abortifacient, expectorant and antimicrobial [1]. Non-glycosidic iridoids like Mussaein, from *Mussaenda pubescens*, are cytotoxic [2]. This report is the first phytochemical and biological investigation on *Mussaenda luteola* Delile shrubs.

Several triterpenoid cycloartane saponins have been isolated from *M. pubescens* [3–5]. Most saponins possess a variety of bioactivities including cardiac, antifungal, hemolytic activities and the ability to affect metabolism and biosynthesis [6]. Mussaendoside F, isolated from *M. pubescens*, was considered as an antagonist of the M-Ach receptor. Additionally, it significantly promoted the proliferation of T-cells in mice in vitro [4]. In the continuation to search for new active metabolites, this paper reported the isolation and structure elucidation of five new saponins from *M. luteola*. Additionally, their antiprotozoal activities and cannabinoid, and opioid receptor binding affinities were evaluated.

Trypanosoma brucei brucei is a unicellular parasite transmitted by the bite of tsetse fly and is the causative agent of sleeping sickness in humans and related diseases in animals [7]. Current treatment of both African and American trypanosomiasis is unsatisfactory [8]. For the treatment of sleeping sickness only four drugs are available [9]. Suramin and pentamidine are effective against the early stages of *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* infections, respectively [10]. Melarsoprol is a trivalent arsenical agent and was introduced in 1949 for treating of late-stage sleeping sickness caused by *T. brucei* spp. [10]. DFMO, a selective inhibitor of ornithine decarboxylase, is the only new drug for chemotherapy of sleeping sickness, which was first used in 1990 [10]. Thus, the identification of new agents with selective trypanocidal activity, that can serve as lead compounds for the development of future antitrypanosomal drugs, is of paramount importance.

2. Experimental

2.1. General procedures

Optical rotations were measured with an Autopol IV automatic polarimeter. IR spectra were obtained using a Bruker Tensor 27 IR

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spectrometer. UV spectra were recorded on Cary-50 Bio spectrophotometer. The ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer at 400 (^{1}H) and 100 (^{13}C) using TMS as internal standard. The HR-ESI-MS were obtained using a Bruker BioApex-FTMS with electrospray ionization (ESI). Column chromatography (CC) was performed on silica gel 60 F254 (0.2 mm, Merck), Diaion HP-20, Sephadex™ LH-20 and MN-polyamide-SC-6.

2.2. Plant material

Aerial parts of *M. luteola* were collected from the El-Zohria Research Garden, Cairo, Egypt in May 2012. The plant material was identified by Professor Mo'men Mostafa Mahmoud, Professor of Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen (No. 36) has been deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Egypt.

2.3. Extraction and isolation

The dried powdered plant material (600 g) was exhaustively extracted by maceration with 70% methanol (4 L × 3) at room temperature for three days. The combined extracts were evaporated under reduced pressure to afford a dry residue (50 g). Silica gel VLC was used for the initial fractionation of the methanolic extract eluted sequentially with *n*-hexane, EtOAc and MeOH to give three fractions (F1–F3). F3 (28.5 g) was subjected to Diaion-HP-20 CC and eluted with distilled water then methanol. The methanol subfraction (7.2 g) was subjected to MN-polyamide-SC-6 CC, which was eluted initially with water then gradient decreased polarities with H_2O–MeOH systems to give eight subfractions (Fr.1–8). Further purification for subfraction Fr.5 (652.6 mg), eluted by H_2O–MeOH (60:40), on silica gel (25 g) CC initially eluted with $CHCl_3$–MeOH (90:10) then (85:15) and (80:20) resulted in the isolation of compound **5** (6.8 mg). Subfraction Fr.6 (490.9 mg), eluted by H_2O–MeOH (1:1), was subjected to Sephadex LH-20 (50 g) CC [1(ID) × 80(L) cm], which was eluted with MeOH to give three subfractions Fr.6-A to Fr.6-C. Fr.6-B (305.6 mg) was subjected to silica gel (10 g) CC [1(ID) × 20(L) cm], which was eluted initially with EtOAc-DCM-MeOH-H_2O (80:40:11:2) to obtain subfractions (1–80) followed by EtOAc–DCM–MeOH–H_2O (15:8:4:1) to obtain subfractions (81–140) to afford compounds **3** (5.4 mg) and **4** (4.7 mg). Subfraction Fr.7 (207 mg), eluted by H_2O–MeOH (40:60), was subjected to Sephadex LH-20 (25 g) CC [1(ID) × 40(L) cm], which was eluted with DCM–MeOH (1:1) to give three subfractions (Fr.7-A to Fr.7-C). Fr. 7A (118.5 mg) was subjected to silica gel (8.0 g) [1(ID) × 20(L) cm] CC, which was eluted initially with $CHCl_3$ followed by gradient $CHCl_3$–MeOH systems (95:5, 90:10 then 85:5) to afford compound **1** (11.0 mg) and compound **2** (15.0 mg).

Heinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (**1**). Yellowish white amorphous powder; $[\alpha]_D^{20}$ + 18.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3330, 2924, 2861, 1767, 1735, 1646, 1069, 1023 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm; 264.0 (4.29), 202.0 (4.10); for ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz) see Tables 1 and 2; HR-ESI-MS *m/z* 1058.5658 $[M + Na]^+$ (calcd. 1058.5664).

Heinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**2**). White amorphous powder; $[\alpha]_D^{20}$ + 6.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3305, 2924, 2871, 1645, 1068, 1025 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm; 264.0 (4.04); for ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz) see Tables 1 and 2; HR-ESI-MS *m/z* 1220.6163 $[M + Na]^+$ (calcd. 1220.6192) and 1196.6216 $[M - H]^-$ (calcd. 1196.6217).

2α-Hydroxyheinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (**3**). White amorphous powder; $[\alpha]_D^{20}$ + 6.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3337, 2924, 2866, 1766, 1649, 1071, 1039, 1023 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm; 262.0 (4.78); for ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz) see Tables 1 and 2; HR-ESI-MS *m/z* 1050.5730 $[M - H]^-$ (calcd. 1050. 5637) and *m/z* 1086.5535 $[M + Cl]^-$ (calcd. 1086. 5404).

2α-Hydroxyheinsiagenin A 3-*O*-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl]-(1→4)]-β-D-glucopyranoside (**4**). Yellowish white amorphous powder; $[\alpha]_D^{20}$ + 10.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3347, 2921, 2889, 1769, 1640, 1069, 1038 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm; 262.0 (3.92); for ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz) see Tables 1 and 2; HR-ESI-MS *m/z* 1090.5656 $[M + Na]^+$ (calcd. 1090.5562) and *m/z* 1066.5856 $[M - H]^-$ (calcd. 1066.5586).

N-(2*S*, 3*R*, 4*R*-3-methyl-4-pentanolid-2-yl)-18-hydroxylanosta-8 (9), 22*E*, 24*E*-trien-27-amide-3-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**5**). Yellowish white amorphous powder; $[\alpha]_D^{20}$ + 10.0 (*c* 0.02, CH_3OH); IR (KBr) ν_{max} 3366, 2926, 1767, 1070, 1046 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm; 261.0 (3.94), 255.0 (3.95); for ^{1}H- and ^{13}C-NMR (C_5D_5N, 400 MHz) see Tables 1 and 2; HR-ESI-MS *m/z* 1236.6196 $[M + Na]^+$ (calcd. 1236.6141).

2.4. Biological activities

2.4.1. Antiprotozoal assay

Compounds **1**–**5** were tested for their antiprotozoal activities against *Leishmania donovani* Promastigote, *L. donovani* Amastigote, *L. donovani* Amastigote/THP1 cells and *T. b. brucei* employing the methods described previously [11]. The in vitro antileishmanial and antitrypanosomal assays were done on cell cultures of *L. donovani* promastigotes, axenic amastigotes, THP1-amastigotes, and *T. brucei* trypomastigotes by Alamar Blue assays as described earlier [11]. The assays have been adapted to 384 well micro-plate format. In a 384 well micro-plate, the samples with appropriate dilution were added to the *L. donovani* promastigotes or *L. donovani* axenic amastigotes or *T. brucei* trypomastigotes cultures (2×10^6 cell/mL). The compounds were tested at three concentrations ranging from 40 to 1.6 μg/mL or 10–0.25 μg/mL. The plates were incubated at 26 °C for 72 h (37 °C for axenic amastigotes and *T. brucei* trypomastigotes) and growth of the parasites in cultures were determined by Alamar Blue assay [11]. The compounds were also tested against *L. donovani* intracellular amastigotes in THP1 cells employing a parasite-rescue and transformation assay [12]. The compounds were simultaneously tested for cytotoxicity against THP1 cell cultures. The conditions for seeding the THP1 cells, exposure to the test compounds and evaluation of cytotoxicity were the same as described in parasite-rescue and transformation assay [12]. IC_{50} and IC_{90} values were computed from the dose response curves using XLfit software. DFMO (difluoromethylornithine) was used as a positive control.

2.4.2. Opioid and cannabinoid receptor binding assay

This screen is designed to use a series of controls to determine the binding affinity of the test compounds using a 96-well format [13]. 10 μM of a positive control [CP-55,940 for cannabinoid receptor binding screen] and [DPDPE (Delta), nor-Binaltorphimine dihydrochloride (Kappa) and DAMGO (Mu) for opioid receptor binding screen] were used to ascertain non-specific binding (NSB) and 1% ethanol or DMSO in Tris-EDTA buffer was used to ascertain total binding. To eliminate the possibility of contamination in the test compounds or controls, wells with 1% ethanol or DMSO with no membrane were tested. Each test well contained 100 μL of the control, 10 μL of test compound, or vehicle and 100 μL cell membrane. Data was analyzed by a non-linear curve fit model using Graph Pad Prism 5.04 software (GraphPad, La Jolla, CA) and IC_{50} values were calculated. The reaction was terminated via rapid filtration with cold Tris–HCl buffer through a UniFilter GF/B 96-well plate pre-soaked with 0.3% BSA. When the filters were dry, 25 μL MicroScint was applied to each filter and the plates were read