Rottlerin Derivatives and Other Compounds from *Mallotus philippinensis* Fruits and Their Potential Antimycobactrial Activity

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Abstract

The methanolic extract of the fruits of *Mallotus philippinensis* afforded 13 compounds, 7,11-diketo-lanost-3-ol (**1**, as acetate), lanosta-8-ene-3β-ol (**2**, as acetate), pregnenolone (**3**, as acetate), *trans*-chalcone (**4**), kamalachalcone E (**5**), oleanolic acid (**6**), gallic acid (**7**), kaempferol (**8**), myricetin (**9**), 1-(5,7-dihydroxy-2,2,6-trimethyl-2 H-1-benzopyran-8-yl)-3-phenyl-2-propen-1-one (**10**), 4′-hydroxyisorottlerin (**11**), rottlerin (**12**), and shikimic acid (**13**). Compound **1** was isolated as a new natural product and its structure was elucidated by 1D and 2D nuclear magnetic resonance analyses including heteronuclear single quantum correlation, heteronuclear multiple-bond correlation, correlation spectroscopy, and nuclear Overhauser effect spectroscopy experiments. All of the isolated compounds were evaluated for their antimycobacterium activity against *Mycobacterium tuberculosis* H37Ra. Compounds **11** and **12** exhibited promising inhibitory activity with IC_{50} values of 0.89 ± 0.33 µg/mL (MIC 2.06 ± 0.41 µg/mL) and 7.59 ± 0.42 µg/mL (MIC 11.56 ± 0.35 µg/mL), respectively.

Key words

Mallotus philippinensis · Euphorbiaceae · antimycobacterium · rottlerins · lanostanol · flavonoids

Supporting information available online at http://www.thieme-connect.de/products

The genus *Mallotus* is a large genus of trees/shrubs distributed chiefly in the tropical and subtropical regions of the Old World with around 20 species in India [1]. The genus *Mallotus* is represented by five species in Maharashtra, and *Mallotus philippinensis* (Lam.) Muell.Arg. (Euphorbiaceae) is a branched tree distributed throughout the state [2]. A red dye called Kamala is secreted on the surface of the fruits and is used medicinally as well as commercially as a dye. It has a purgative property and is also used in the external applications for parasitic infections of the skin. It also has a lithontriptic property that dissolves or destroys stones in the kidneys and it is styptic (antihemorrhagic agent). Earlier work on the chemical analysis of different parts of this tree has revealed the occurrence of several triterpenes [3,4], flavonoids [5–8], and dimeric chalcone derivatives [9,10].

In our earlier work, from the acetone extract of whole uncrushed fruits of *M. philippinensis*, we isolated chalcones, including one new dimeric chalcone, kamalachalcone E, which was shown to have antifungal activity against the human pathogens *Candida albicans, Candida glabrata*, and *Cryptococcus neoformans* [11]. In the continuation of this work, we have examined the methanol extract of the crushed fruits of *M. philippinensis* to isolate 13 compounds (**1**–**13**) and evaluated them for antimycobacterium activity against *Mycobacterium tuberculosis* H37Ra.

Compound **1** was isolated as an acetate. Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) showed pseudomolecular peaks at 501.02 $[M+1]^+$ and 539.01 $[M+K]^+$, corresponding to the molecular formula $C_{32}H_{52}O_4$ with seven degrees of freedom. The ^{1}H NMR (**Fig. S1**, Supporting Information) spectrum showed five singlet methyls at δ_H 0.72, 0.84, 0.92, 1.21, 1.30, one three proton doublet methyl at δ_H 0.85 (3 H, d, 6.0 Hz), one six proton doublet methyl at 0.87 (6 H, d, 6.7 Hz), and seven methines at δ_H 1.35, (m), 1.53 (sp, 6.9 Hz), 1.60 (m), 1.26 (m), 2.22 (d, 12.9 Hz), 2.67 (d, 12.7 Hz), and 4.51 (dd, 7.6, 5.6 Hz). The ^{13}C NMR (**Fig. S2**, Supporting Information) and distortionless enhancement by polarization transfer (DEPT, **Fig. S3**, Supporting Information) spectra showed the presence of eight methyls, nine methylenes, seven methines, and six quaternary carbons, including two carbonyl groups at δ_C 209.0 and 209.6. This data suggests that compound **1** was a tetracyclic triterpenoid belonging to the class lanostanol. The complete structure was elucidated by 2D NMR. A proton at δ_H 2.59 (H-12) showed an HMBC correlation with the carbonyl carbon at δ_C 209.6 (C-11), while a proton at δ_H 1.26 (H-5) showed an HMBC correlation with the carbonyl carbon at δ_C 209.0 (C-7). The key HMBC correlations are shown in **Fig. S4**, Supporting Information. NOESY correlations were observed between H_3-18 (δ_H 0.72) and H-8 (δ_H 2.67), between H_3-19 (δ_H 1.30) and H-8 (δ_H 2.67), H_3-29 (δ_H 0.91), and between H_3-21 (δ_H 0.85) and H-9 (δ_H 2.22), which led to the assignment of the relative stereochemistry (**Fig. S4**, Supporting Information). Thus, **1** was identified as 7,11-diketo-lanost-3β-ol. It has been prepared synthetically and reported earlier [12]. To the best of our knowledge, this is the first report of the isolation of compound **1** as a new natural product.

Compounds **2**–**13** were identified as lanosta-8-ene-3β-ol (**2**, as acetate) [13], pregnenolone (**3**, as acetate) [14], *trans*-chalcone (**4**), kamalachalcone E (**5**) [11], oleanolic acid (**6**) [15], gallic acid (**7**) [16], kaempferol (**8**) [17], myricetin (**9**) [18], 3.3.2 1-(5,7-dihydroxy-2,2,6-trimethyl-2H-1-benzopyran-8-yl)-3-phenyl-2-propen-1-one (**10**) [11], 4′-hydroxyisorottlerin (**11**) [10], rottlerin (**12**) [11], and shikimic acid (**13**) [19] (**Fig. 1**) by comparison with the literature NMR data and were confirmed by the LC-ESI-MS. An attempt has been made to define the configuration of compound **11** by comparing it with structurally similar flavanone derivatives. Compound **11** was determined to have a negative specific rotation, $[\alpha]_D^{26}$ − 12.11 (*c* 0.6, chloroform). Literature analysis suggests that the negative specific rotation was consistent with the α orientation of the group at position 2 [20–22]. This allowed for the determination of the α orientation of the group at position 2 in compound **11**. Compounds **1**–**13** were evaluated for their antimycobacterium activity against *M. tuberculosis* H37Ra. Compounds **11** and **12** exhibited antimycobaterium activity with IC_{50} values of 0.89 ± 0.33 µg/mL (MIC 2.06 ± 0.41 µg/mL) and 7.59 ± 0.42 µg/mL (MIC 11.56 ± 0.35 µg/mL), respectively, as compared to the positive control rifampicin.

Materials and Methods

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III Ultra Shield NMR (^{1}H operating frequency: 400 MHz) instrument. LC-ESI-MS was recorded with the Waters Acquity LC-MS instru-

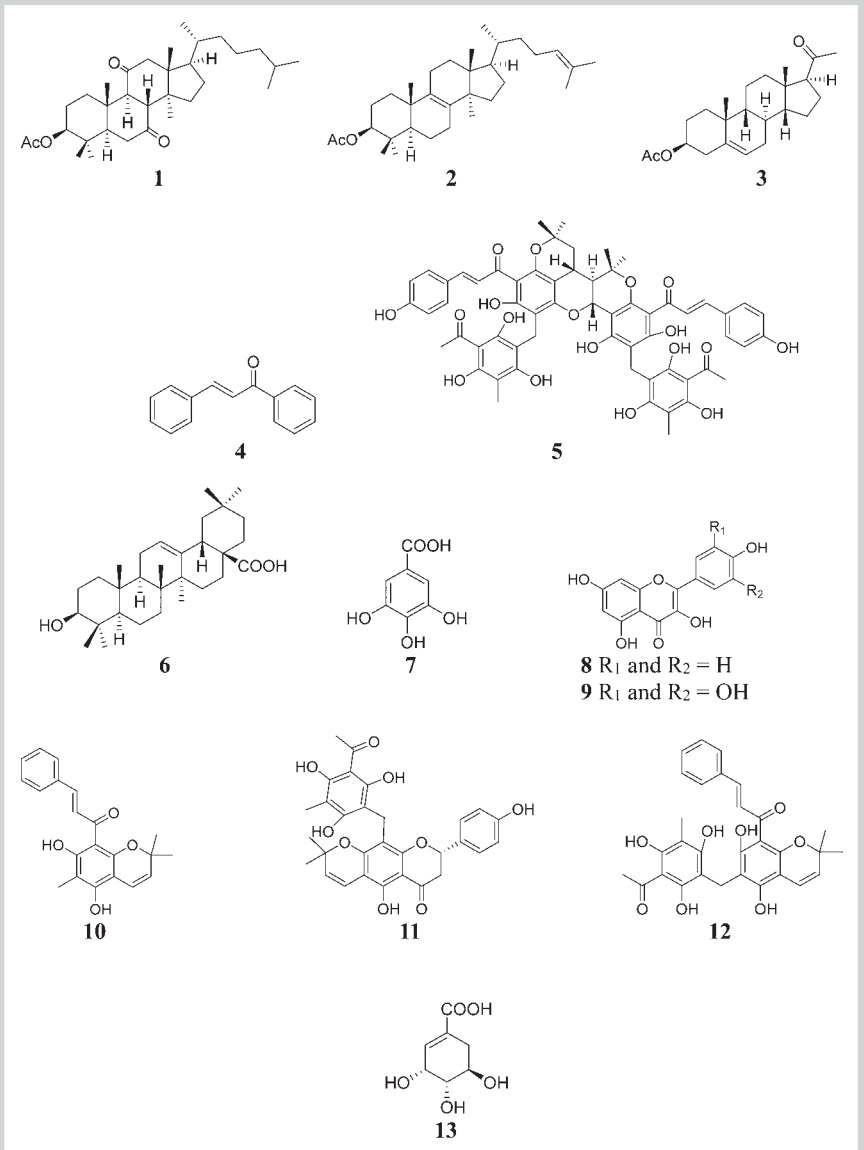

Fig. 1 Compounds isolated from the methanol extract of *M. philippinensis* fruits.

ment. Optical rotations were measured with a JASCO P-1020 polarimeter. The Spectramax plus384 plate reader (Molecular Devices, Inc.) was used. All the solvents used were distilled prior to use. Column chromatography (CC) was performed with silica gel purchased from Thomas Baker Ltd. Preparative thin-layer chromatography (TLC) was carried out using TLC plates supplied by Merck Ltd. The *M. tuberculosis* H37Ra (ATCC 25 177) strain was obtained from MTCC, Chandigarh, India. Rifampicin (97.0 %), XTT sodium salt, and menadione were purchased from Sigma-Aldrich.

Plant material

Fruits of *M. philippinensis* were collected from Bhimashankar Forest area, Pune District in March 2012. A herbarium voucher is deposited at Botanical Survey of India, Western Circle, Pune (No. PCOPAVMA2).

Extraction and isolation

Whole fruits obtained after acetone extraction were dried under airflow and pulverized. The pulverized fruit powder, 1.2 kg, was extracted with methanol by a cold maceration technique (3 L × 3 × 14 h) at room temperature. After solvent evaporation at a reduced pressure, a red-brown extract (31.0 g, 2.58 %, based on dry weight) was yielded. The methanol extract, 30 g, was separated by repeated CC on silica gel with different elution systems. The final purification of the compounds was achieved by preparative TLC. The detailed extraction and isolation of the compounds **1**–**13** as well as LC-ESI-MS, UV, IR, and NMR data of the compounds are available as Supporting Information.

Antimycobacterium bioassay using the XTT reduction menadione assay

M. tuberculosis H37Ra cells (ATCC 25 177) were grown to the logarithmic phase ($O.D._{59}$ 5 ~ 1.0) in M. pheli medium. Compounds **1**-**13** were dissolved in dimethyl sulfoxide and stored in aliquots at − 20 °C. A freshly prepared stock solution (1.25 mM) of XTT sodium salt in sterile 1 x PBS and 6 mM menadione in DMSO was used. Compounds were screened for their inhibitory effect on *M. tuberculosis* by following the XTT aeduction menadione assay (XRMA) protocol [23,24]. Briefly, in all wells of the assay plate, 200 µM XTT were added and incubated at 37 °C for 20 min. Menadione, 60 µM, was added and incubated at 37 °C for 40 min. The

optical density was recorded on a microplate reader at 470 nm (filter) against a blank prepared from cell-free wells. Absorbance given by the cells treated with the vehicle alone was taken as 100% of cell growth. All the experiments were performed in triplicate and the quantitative values are expressed as the average ± standard deviation, and the IC_{50} values were calculated from their dose-response curves (**Figs. S5** and **S6**, Supporting Information). Compounds **11** and **12** exhibited promising inhibitory activity with IC_{50} values of 0.89 ± 0.33 µg/mL (MIC 2.06 ± 0.41 µg/mL) and 7.59 ± 0.42 µg/mL (MIC 11.56 ± 0.35 µg/mL), respectively, as compared to that of positive control rifampicin with an IC_{50} value of 0.0019 ± 0.0003 µg/mL (MIC 0.02 ± 0.31 µg/mL).

Supporting information

^{1}H, ^{13}C, DEPT, and 2D correlations of compound **1**, dose-response curves for compounds **11** and **12** against *M. tuberculosis* H37Ra, and the extraction, isolation, NMR, and other characterization data of compounds **1**–**13** are available as Supporting Information.

Acknowledgments

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Conflict of Interest

There is no conflict of interest among all authors.

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