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Carbazole alkaloids with antiangiogenic activities from *Clausena sanki*

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ABSTRACT

Two new carbazole alkaloids **1** and **2**, and eleven known congeners **3**–**13** were isolated and identified from *Clausena sanki* for the first time. Their structures were elucidated on the basis of extensive UV, IR, MS, NMR spectroscopic data and comparison with literatures. The compounds **1**–**13** were evaluated by MTT assay to determine whether they decreased VEGF-mediated cell proliferation in HUVECs with Axitinib as positive control. Among them, compounds **1**, **2**, **6**, **8**, and **13** (μM) exhibited moderate antiangiogenic activities, which inhibited VEGF-induced HUVEC proliferation *in vitro* with IC_{50} values of 12.1 (C.I. 8.2–15.2), 58.1 (C.I. 56.3–63.4), 13.7 (C.I. 9.2–15.4), 16.0 (C.I. 9.5–16.4), and 63.2 (C.I. 57.8–65.7) μM, respectively. Moreover, the antiangiogenic activities of compounds **1**–**13** were evidenced *in vivo* in the zebrafish embryo model. As a result, compounds **1**, **2**, **6**, **8**, and **13** showed effectively suppress angiogenesis. These research results may guide the search for new natural products with antiangiogenic attributes.

1. Introduction

Clausena anisum-olens (Blanco) Merr. is a synonym of *Clausena sanki* (Perr.) Molino which belongs to the Rutaceae family. It is a shrub growing wild and cultivated from Philippines and South China in Southeast Asia [1]. It is not only used as a condiment, but also a multipurpose traditional medicinal herb for treating dysentery and arthritis for hundreds of years [2]. Meanwhile, it also has the functions of dredging intestines and stomach, relieving dyspepsia, and promoting digestion [3,4]. Different plant parts have biological activities which are antibacterial [4], tumor-promotion inhibitory [5], and antioxidant [6] activities. Previous phytochemical studies have indicated that *C. sanki* contained coumarins [5], cyclopeptides [7], and essential oils [2–4].

To the best of our knowledge, there have been only a few reports on antiangiogenic activities about *C. sanki*, and its antiangiogenic constituents as well as its mechanism of action are worthy of further exploring and studying. Therefore, we carried out a bioassay-guided investigation of *C. sanki* in order to evaluate its antiangiogenic activities. Modern pharmacological research showed that some carbazole alkaloids possessed antiangiogenic activities [8–10]. In our previous work, the $CHCl_3$ extract (active fraction) of *C. sanki* was revealed to display antiangiogenic activity, which prompted us to study its further active components. As a result, thirteen carbazole alkaloids **1**–**13** including two novel structures, named 6,7′-dimethoxy-3,3′,13,13′,14,14′-hexamethyl-9,9′-dihydro-[5,5′-bipyrano-carbazole]-6′,7-diol (**1**) and 1,9-dimethoxy-3-methyl-9*H*-carbazol-2-ol (**2**), were isolated and identified from *C. sanki* on the basis of extensive spectroscopic analysis and comparison with references (Fig. 1). Moreover, the known compounds **3**–**13** were isolated from *C. sanki* for the first time. Meanwhile, the isolates **1**–**13** of *C. sanki* were evaluated for their antiangiogenic activities in this work. These research results may guide the search for new natural products with antiangiogenic attributes.

2. Materials and methods

2.1. General experimental procedures

The melting points were recorded on a XT5B microscopic melting point apparatus which was uncorrected. The optical rotations were measured on a Perkin-Elmer 241 digital polarimeter at 20 °C. The UV and IR spectra were measured by Australia GBC UV-916 spectrophotometer and Nicolet 5700 FT-IR spectrometer with KBr pellets, respectively. The 1D and 2D NMR spectral data were run on Bruker-400 with TMS as internal standard [11]. The ESI-MS data were recorded by using a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer, and the HR-ESI-MS data were

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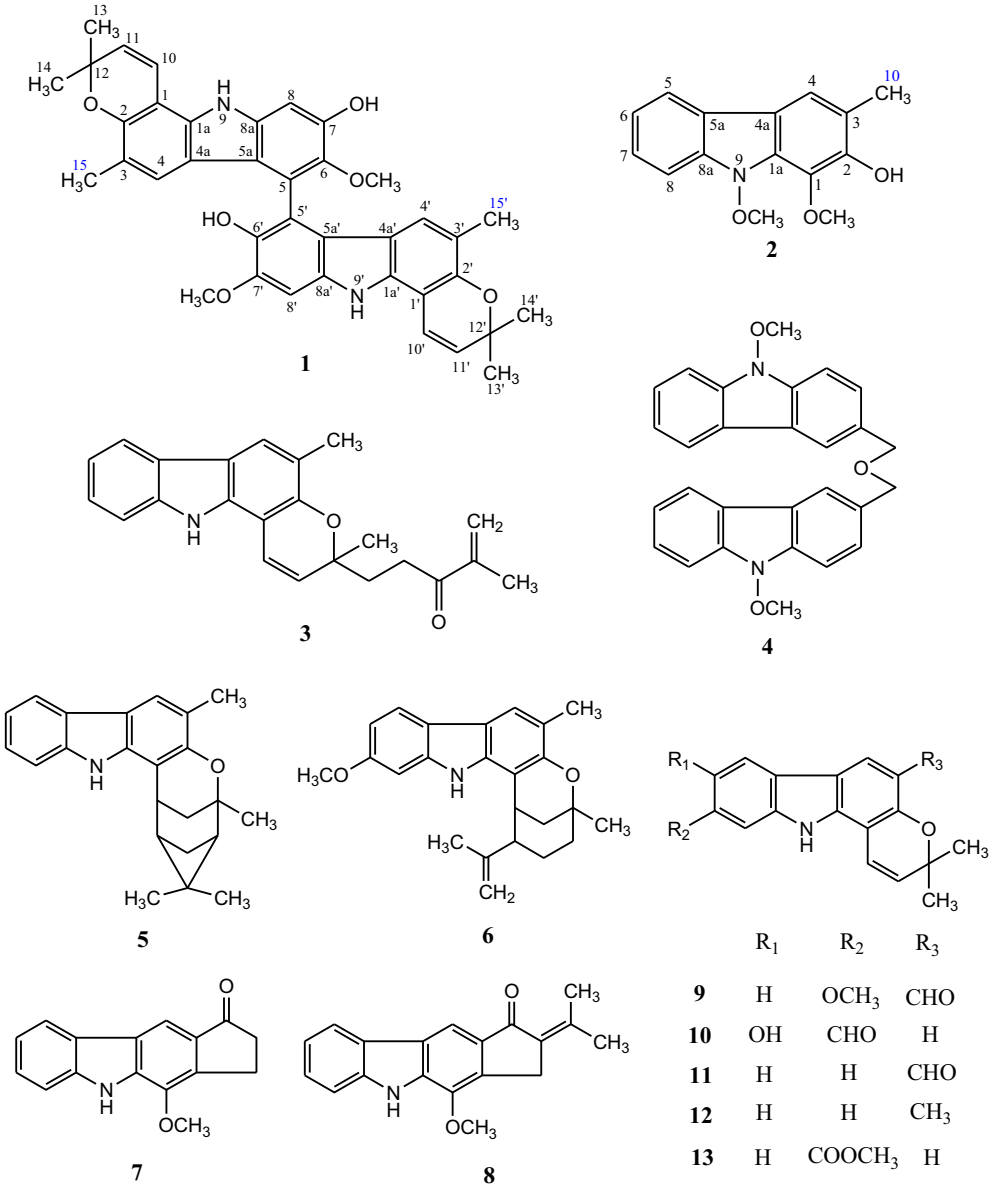

Fig. 1. Structures of compounds **1**–**13.**

measured by using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. The Reversed-phase HPLC was performed using Agilent 1200 series with a DIKMA (4.6 × 250 mm) analytical column packed with C18 (5 μm) [12]. The column chromatography was carried out on silica gel H, 100–200 mesh, 200–300 mesh, Sephadex LH-20, and Toyopearl HW-40. The TLC was performed on precoated silica gel GF254 plates and the spots were visualized under UV light (254 or 365 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating [13].

2.2. Plant material

The whole plant of *Clausena sanki* were harvested from Maguan County, Yunnan province, China, in October 2015. This plant was identified by Dr. Su Zhang of Wuyang Weisen Biological Medicine Co., Ltd., The voucher specimen (NO. HP-201510) of *C. sanki* has been deposited in Nanyang Normal University, Nanyang 473061, China.

2.3. Extraction and isolation

The dried leaves and twigs of *C. sanki* (15.0 kg) were extracted with 95% EtOH (38 L × 3) heating under reflux for 3 h each time. The extracts were concentrated by rotary evaporator under reduced pressure resulting in a dark black residue (1.3 kg). The combined extracts were successively suspended in 1.5% HCl (4500 mL) and set aside for 24 h. The filtrate was adjusted with ammonia into pH = 8.5–9.5, then partitioned with $CHCl_3$ (3 × 400 0 mL), respectively. The active extract of $CHCl_3$ soluble fraction (32.50 g) was performed on the silica gel column (silica gel, 100–200 mesh, 150 g, 3.5 × 60 cm) and eluted with a gradient elution of petroleum ether/EtOAc (15:1 → 10:1 → 8:1 → 6:1 → 4:1 → 2:1) to obtain six fractions: A (3.09 g), B (6.12 g), C (7.75 g), D (5.20 g), E (3.58 g), and F (2.52 g).

The fraction B was chromatographed on silica gel column (silica gel, 200–300 mesh, 60 g, 2.5 × 50 cm) eluting with petroleum ether/EtOAc (12:1 → 10:1 → 8:1) to obtain three sub-fractions: B-a (1.68 g), B-b (2.24 g), B-c (1.26 g). The separation of B-b (2.24 g)