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Solid Tumor Inhibitory and other Constituents of *Casimiroa tetrameria*

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[ABSTRACT] AIM: To isolate and characterize solid tumor inhibitory and other constituents from a bioactive extract of *Casimiroa tetrameria* ((Rutaceae). **METHODS:** A crude extract of *C. tetrameria* obtained from the US National Cancer Institute Natural Product Repository and found to exhibit selective toxicity to solid tumor cells was subjected bioactivity-guided fractionation involving solvent-solvent partitioning, gel filtration, and chromatography. The structures of all isolated compounds were elucidated by spectroscopic analysis (NMR and MS) and/or by comparison with the reported data. Compounds **1** and **4**–**9** were evaluated for their solid tumor selective cytotoxicity. **RESULTS:** Nine metabolites, including a new furanocoumarin, 5-methoxy-8-(4'-acetoxy-3'-methylbut-2-enyloxy)-psoralen (**1**), and the previously known compounds **2**–**9** were encountered. Of these the flavonoid zapotin (**6**), and *N*-benzoyltyramide derivatives **7** and **8** were found to be the active constituents. **CONCLUSION:** Zapotin (**6**) is the most potent constituent of *C. tetrameria* with solid tumor selectivity.

[KEY WORDS] *Casimiroa tetrameria*; Rutaceae; Solid tumor selectivity; Flavonoids; Zapotin; Furanocoumarins; *N*-benzoyltyramides

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1 Introduction

Currently available modalities for the chemotherapy of cancer suffer from the unavailability of drugs to treat solid tumors and the concurrent metastatic disease [1-2]. This is partly because the majority of cell-based antitumor drug discovery efforts that leading to standard chemotherapeutic agents have relied frequently on their potency (cytotoxicity) rather than selectivity. To address this deficiency, we have developed a high-throughput, cost-efficient, and simple end-point disk diffusion soft agar assay based on differential clonogenic cytotoxicity between solid tumor cells and either normal or leukemia cells [3]. Solid tumor selectivity criteria in this approach incorporate both the cellular and molecular targets associated with the cancer phenotype. In our continuing search for anticancer agents with solid tumor selectivity [4], we investigated an extract of *C. tetrameria* (N074701) obtained from the Natural Products Repository of US National Cancer Institute (NCI.) and found that it exhibited selective inhibition of murine solid tumor cell line, Colon 38, compared with murine leukemia cell line, L1210, and human hematopoietic progenitor cells, CFU-GM. Bioassay-guided fractionation resulted in the isolation of nine compounds including three furanocoumarins **1**–**3**, of which **1** is new, three flavonoids **4**–**6**, two *N*-benzoyltyramides **7**–**8**, and an alkaloid **9**. All isolated compounds were evaluated for their solid tumor selectivity. Here we report the isolation of **1**–**9**, structure elucidation of **1**, and solid tumor selective activity of the flavonoid, zapotin (**6**).

Plants of the genus *Casimiroa* (Rutaceae) are distributed in the tropical and subtropical areas of Central America including Mexico. The fruits and leaves of *Casimiroa* species find their applications in folk medicines as sedatives and treatments for dermatological conditions [5]. *C. edulis* is an economically important plant as it produces an edible fruit known as white sapote. The pharmacological studies of an aqueous extract of the seeds of *C. edulis* have confirmed the

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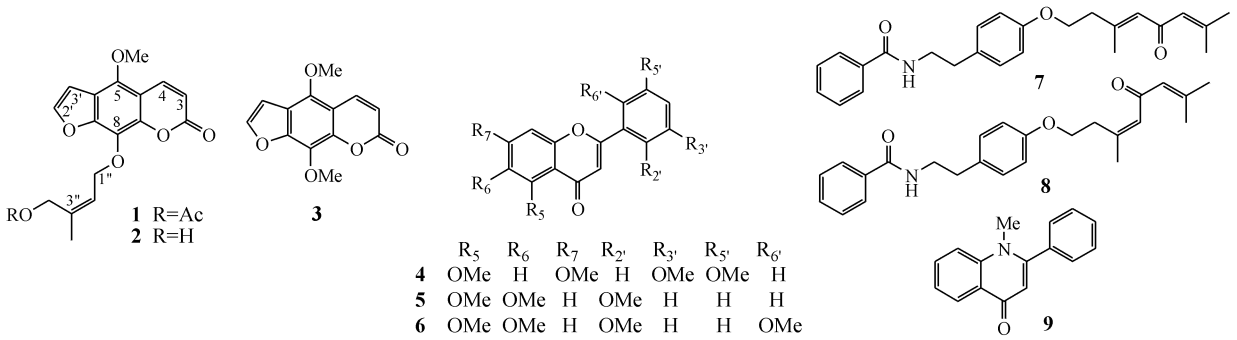

Fig. 1 Structures of compounds 1–9 from *C. tetrameria*

cardiovascular [6-8], muscle relaxant and contractile activities [9]. The sedative effects of an alcoholic extract of the seeds of *C. edulis* in mouse and rat models were also reported [10]. The chemical studies of *C. edulis* have resulted in the isolation of flavonoids, furocoumarins, and alkaloids [11-15], some with anti-mutagenic activity [15]. An ethanolic extract of *C. edulis* has shown cytotoxic activity against human carcinoma cell lines, U251 (brain tumor) and MCF-7 (breast cancer) [14].

2 Experimental

2.1 *General procedures*

UV data were recorded with a Shimadzu UV-1601 instrument. ^{1}H and ^{13}C NMR spectra were measured on Bruker DRX-500 and DRX-600 NMR spectrometers. High resolution and low resolution mass spectra were recorded on an IonSpec FT and Shimadzu LCMS-8000QPα mass spectrometers, respectively. Sephadex LH-20 was purchased from Amersham Biosciences. Analytical HPLC involved a Hitachi L-6200A system equipped with a Hitachi L-4500 photodiode array and Shimadzu ELSD-LT detectors whereas for preparative HPLC a Waters Delta Prep 400 HPLC system equipped with a Waters 996 PDA detector was used. Kromasil C_{18} reversed phase column (250 mm × 10 mm, 5 μm) for HPLC was obtained from Phenomenex Inc.

2.2 *Plant extract*

The crude CH_2Cl_2–MeOH (1 : 1) extract of C. tetrameria was obtained from NCI Natural Products Repository at the Frederick Cancer Research and Development Center (Frederick, Maryland, USA). Sample number N074701 was assigned to this extract.

2.3 *In vitro disk diffusion assay for cytotoxicity*

The disk diffusion assay was used to define differential cell killing among human and murine normal and malignant cell types as previously reported [3-4]. Since the initial extract (N074701) obtained from NCI was found to demonstrate selectivity between murine Colon38 and leukemia L1210 cells, these cell lines were used for bioassay-guided fractionation and evaluation of the active compounds. Both the magnitude of the zonal difference as well as the potency was used for prioritization of fractions.

2.4 *Fractionation and isolation*

The crude extract N074701 (10.2 g) exhibiting solid tumor selective activity in our in vitro disk diffusion assay [3-4] was subjected to solvent-solvent partitioning with hexanes–80% MeOH and $CHCl_3$–50% MeOH to yield hexanes, $CHCl_3$, and 50% MeOH fractions. Of these, only the $CHCl_3$ fraction (1.1 g) was found to be active and was further fractionated on a column of Sephadex LH-20 by successive elution with CH_2Cl_2/hexanes (4 : 1), CH_2Cl_2/acetone (3 : 2), CH_2Cl_2/acetone (1 : 4), and MeOH. The CH_2Cl_2/hexanes (4 : 1) fraction (391.2 mg) which selectively inhibited the growth of solid tumor cells, was separated by semi-preparative HPLC using a Kromasil C_{18} reversed phase column (250 mm × 10 mm, 5 μm) and elution with a gradient (60%–100%) of aqueous MeOH. Detection with UV (230 nm) and evaporative light scattering indicated 8 major peaks which were collected individually. These fractions were then purified by preparative TLC on silica gel to afford compounds **1** (1.9 mg), **2** (0.9 mg), **3** (0.6 mg), **4** (2.5 mg), **5** (8.5 mg), **6** (11.6 mg), **7** (1.6 mg), **8** (2.4 mg), and **9** (2.1 mg).

5-Methoxy-8-(4'-acetoxy-3'-methylbut-2-enyloxy) psoralen (**1**): amorphous yellow powder. UV (MeOH) λ_{max}: 207, 249, 266, 305 nm. Positive HRESI-MS *m/z* 381.096 1 (Calcd. 381.0950 for $C_{19}H_{18}O_7$·Na, $[M + Na]^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.27 (1 H, d, *J* = 9.8 Hz, H-3), 8.10 (1 H, d, *J* = 9.8 Hz, H-4), 7.60 (1 H, d, *J* = 2.3 Hz, H-2'), 6.97 (1 H, d, *J* = 2.3 Hz, H-3'), 4.90 (2 H, d, *J* = 6.9 Hz, H-1"), 5.84 (1 H, br t, *J* = 6.9 Hz, H-2"), 1.79 (3 H, d, *J* = 1.0 Hz, Me-4"), 4.62 (2 H, s, H-5"), 2.02 (3H, s, OAc-5"), 4.16 (3H, s, OMe-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.4 (C-2), 112.9 (C-3), 139.3 (C-4), 144.5 (C-5), 114.6 (C-6), 150.5 (C-7), 126.6 (C-8), 107.6 (C-4a), 144.2 (C-8a), 145.1 (C-2'), 105.1 (C-3'), 69.3 (C-1"), 125.2 (C-2"), 136.5 (C-3"), 21.4 (C-4"), 62.8 (C-5"), 170.8 ($CH_3\underline{C}O$), 20.9 ($\underline{C}H_3CO$), 60.8 (OMe-5).

Acetylation of **2**: Compound **2** (0.2 mg) dissolved in pyridine (100 μL) was treated with Ac_2O (50 μL) and allowed to stand overnight. The reaction mixture was then poured into ice water and extracted with $CHCl_3$, and the $CHCl_3$ extract was dried and concentrated. The product obtained was shown to be identical with **1** by TLC and HPLC comparison.