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Compounds from *Simarouba berteriana* which inhibit proliferation of *NF1*-defective cancer cells

Krishna P. Devkota^a, Jennifer A. Wilson^a, Curtis J. Henrich^{a,b}, James B. McMahon^a, Karlyne M. Reilly^c, and John A. Beutler^{a,*}

Krishna P. Devkota: krishna.devkota@nih.gov; Jennifer A. Wilson: wilsonje@mail.nih.gov; Curtis J. Henrich: henrichcj@mail.nih.gov; James B. McMahon: mcmahoja@mail.nih.gov; Karlyne M. Reilly: reillyk@mail.nih.gov; John A. Beutler: beutlerj@mail.nih.gov

^aMolecular Targets Laboratory, Center for Cancer Research, Frederick, Maryland 21702, United States

^bSAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702, United States

^cMouse Cancer Genetics Program, Center for Cancer Research, Frederick, Maryland 21702, United States

Abstract

A neurofibromatosis type 1 (*NF1*) based bioassay-guided phytochemical investigation on *Simarouba berteriana* led to the isolation of one new canthin-6-one-9-methoxy-5-*O*- β -D-glucopyranoside (**1**), seven known canthine alkaloids (**2–8**), two known quassinoids (**9–10**) and a known neo-lignan (**11**). The structures of all compounds were established by HRMS, 1D- and 2D-NMR analysis and comparison with previously reported data. Most of the compounds inhibited the proliferation of an *Nf1*- and *p53*-deficient mouse glioma cell line at non-cytotoxic concentrations.

Keywords

Simarouba berteriana; Neurofibromatosis type 1; canthine alkaloids; growth inhibition; glioblastomas

1. Introduction

Neurofibromatosis type 1 (*NF1*), von Recklinghausen disease, is one of the most common autosomal dominant disorder that affects one in 3000 livebirths (Huson et al., 1989). *NF1* patients develop a diverse set of tumor types, including benign neurofibromas, malignant peripheral nerve sheath tumors (MPNST), gliomas, gastrointestinal stromal tumors, myeloid leukemia, breast cancer and others (Brems et al., 2009). Genetic alterations can occur in Ras genes, upstream regulators, or downstream effectors (McGillicuddy et al., 2009). The protein associated with *NF1*, neurofibromin, is involved in the downregulation of the rat sarcoma viral oncogene homologue (RAS)-mitogen activated protein kinase (MAPK) pathway and *NF1* belongs to the group of RAS-MAPK disorders (Brems et al., 2009). We

*Corresponding author. Tel: +1 301-846-1942; Fax: +1 301-846-6177.

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used a recently developed screen (Hawes, et al., 2008) to identify natural product extracts which inhibit the growth of *NF1*-defective tumor cell proliferation. This paper describes *NF1*-assay guided isolation and structure elucidation of compounds obtained from *Simarouba berteriana* Krug & Urban.

S. berteriana belongs to the plant family Simaroubaceae which contains 32 genera and over 170 arboreal or shrubby species (Fo et al., 1992). Canthin-6-one and canthin-6-one-3-*N*-oxide are common constituents of this family which possess significant biological properties including cytotoxicity (Rivero-Cruz et al., 2005; Kardono et al., 1991; Jiang and Zhou, 2011; Kuo et al., 2003), antimalarial (Kuo et al., 2003), antifungal (Li et al., 1993), phosphodiesterase inhibition (Ohmoto et al., 1988) and antiviral (Ohmoto et al., 1985). Quassinoids, another important class of compounds from this family have been extensively investigated for useful biological activities such as anti-leukemic (Yoshimura et al., 1985), antitumor (Usami et al., 2010) and antimalarial (Kardono et al., 1991). However, no investigation has so far been conducted on the plants of this family with respect to their effect on *NF1*-deficient cancer cells. In the current study, we have isolated eight canthine alkaloids (**1–8**), two quassinoids (**9–10**) and a neo-lignan (**11**) from *S. berteriana* active against *NF1* deficient cancer cells.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. The molecular formula, $C_{21}H_{20}N_2O_8$, was determined by its HRMS. The IR spectrum showed the presence of an hydroxyl (3415 cm^{-1}) and a lactam carbonyl (1650 cm^{-1}). The UV spectrum with absorption maxima at 205 (4.02), 241 (4.23), 274 (4.11), 315 (3.89), 348 (4.01), and 365 (3.98) nm suggested that compound **1** had a canthin-6-one chromophore (Li et al., 1993; Jiang and Zhou, 2011). The ^1H -NMR spectrum of **1** (Table 1) showed a pair of *ortho*-coupled signals at δ 8.07 (1H, d, $J = 5.0\text{ Hz}$) and 8.73 (1H, d, $J = 5.0\text{ Hz}$) which were assigned to H-1 and H-2, respectively. A downfield singlet resonated at δ 7.59 exhibiting HMBC correlations (Table 1) with C-5 (δ 151.7), C-6 (δ 154.7), C-15 (δ 127.6) and C-16 (δ 135.5) was assigned to H-4. The ABX type signals at δ 8.08 (1H, d, $J = 2.2\text{ Hz}$), 7.21 (1H, dd, $J = 8.6, 2.2\text{ Hz}$) and 8.27 (1H, d, $J = 8.5\text{ Hz}$) corresponded to the three aromatic protons H-8, H-10 and H-11, respectively of the monosubstituted indole moiety. The monosubstitution at C-9 was a methoxy moiety which resonated at δ 3.95 (3H, s) showing an HMBC correlation with C-9 (δ 161.6). The presence of a glucopyranoside moiety in compound **1** was confirmed by the ^1H and ^{13}C signals in the NMR spectrum (Table 1). This glycoside was assigned at the C-5 position due to the observed HMBC correlation of the anomeric proton H-1' (δ 5.21) with C-5 (δ 151.7). The ^{13}C -NMR data of **1** (Table 1) is consistent with the canthin-6-one skeleton (Fo et al., 1992; Kanchanapoom et al., 2001; Ouyang et al., 1994). Based upon these arguments, compound **1** was assigned as a new compound named canthin-6-one-9-methoxy-5-*O*- β -D-glucopyranoside.

Together with a new canthin-6-one, seven known canthine alkaloids **2–8**, two known quassinoids **9–10** and a known neo-lignan **11** were also isolated and characterized by comparing the spectral data with the literature. The known compounds are 9-methoxycanthin-6-one (**2**) (Ohmoto et al., 1976), 8-hydroxy-9-methoxycanthin-6-one (**3**) (Fo et al., 1992), 9-hydroxycanthin-6-one (**4**) (Fo et al., 1992), canthin-6-one-3-*N*-oxide (**5**) (Ohmoto et al., 1976), 9-hydroxycanthin-6-one-*N*-oxide (**6**) (Kardono et al., 1991), 11-hydroxycanthin-6-one-*N*-oxide (**7**) (Ouyang et al., 1994), β -carboline-1-propionic acid (**8**) (Ohmoto and Koike, 1984), ailanqyassin (**9**) (Aono et al., 1994), glaucarubinone (**10**) (Usmai et al., 2010) and balanophonin (**11**) (Haruna et al., 1982).

All of the purified compounds were tested in an *Nf1*- and *p53*-defective mouse malignant glioma tumor cell line engineered to express a dual reporter, in which cell viability and growth inhibition are reported separately in the same cells using filtered red and green luminescence, respectively (Table 2). Loss of cell viability is measured using a red luminescent reporter of all living cells comparing compound-treated cell in growth media relative to untreated controls in growth-retarding media. Growth inhibition is measured using a green luminescent reporter only expressed in actively dividing cells comparing compound treated cells in growth media to vehicle treated cells in growth media. High growth inhibition scores indicate that cells stop dividing in the presence of the compound.

The cytotoxicity and inhibition of cell proliferation varied clearly with the class of compounds (Table 2). The quassinoid class of compounds (**9** and **10**) showed the best activity among other class of compounds with cell proliferation inhibition of 71 percent and 57 percent, respectively, at 2 $\mu\text{g/mL}$ without observed toxicity. The neo-lignin compound **11** showed activity weaker than the quassinoids but stronger than the canthine alkaloids **1–8**. The majority of the canthine alkaloids exhibited moderate inhibition of cell growth with some toxicity. It should be noted that the same extract was previously identified as a potential AP-1 inhibitory extract, and though quassinoids were not purified from this species in that project, they were considered likely to be responsible for the observed AP-1 inhibition (Beutler, et al., 2009).

3. Experimental

3.1. General

Optical rotations ($[\alpha]_D$) were measured on a Perkin-Elmer 241 polarimeter in a 100 x 2 mm cell (units $10^{-1} \text{ deg cm}^2\text{g}^{-1}$). UV absorption spectra were obtained using a Varian Cary 50 Bio UV-visible spectrophotometer. IR spectra were measured using a Perkin Elmer FT-IR Spectrometer, SPECTRUM 2000. LCMS were obtained using a Hewlett Packard Series 1100 MSD, whereas HRMS were acquired on an Agilent 6520 Accurate Mass Q-TOF instrument with internal reference masses at 121.05087 and 922.00979, both within 5 ppm. The NMR experiments were performed on a Bruker 600 MHz NMR spectrometer. ^1H and ^{13}C spectra were referenced to deuterated solvent peaks. The diol DIO Spe-ed SPE cartridge and Sephadex LH-20 columns attached to a model UA-6 UV detector and Foxy 200 fraction collector (Teledyne Isco) were used for fractionation of the extract, whereas purification of the compounds was performed using a Varian ProStar 210/215 HPLC equipped with a Varian ProStar 325 UV-Vis detector, operating under Star 6.41 chromatography workstation software. All solvents and chemicals were of analytical grade.

3.2. Plant material

Stem bark of *Simarouba berteriana* Krug & Urban was collected in the Dominican Republic, Province of Peravia, east of poblado de Salinas, $18^{\circ}13'\text{N}$, longitude $-70^{\circ}55'\text{W}$, 5 m altitude, by R. Garcia and J. Pimentel, April 2, 1988 under the auspices of the New York Botanical Garden. A voucher specimen sheet#3271881 is deposited in the US National Herbarium.

3.3. Extraction and isolation

Dried, ground stem bark (489 g) was extracted by the standard NCI procedure (McCloud, 2010), with a yield of organic soluble extract of 20.77 g. An aliquot (2.3 g) of this organic extract was loaded on diol DIO Spe-ed SPE cartridges (230 mg/cartridge) and eluted with 6.0 mL each of hexane/ CH_2Cl_2 (9:1), $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (20:1), EtOAc, EtOAc/MeOH (5:1) and MeOH to give five fractions A-E of 380.9 mg, 512.0 mg, 218.6 mg, 565.0 mg and 304.1 mg, respectively. The activity against *NF1*-deficient cells of fractions B and D was higher

than other fractions, thus these were selected for further fractionation and isolation of pure compounds. Fraction B (470.0 mg) was chromatographed on a 2.5 x 40 cm Sephadex LH-20 column and eluted with hexane/CH₂Cl₂/MeOH (2:5:1, v/v), with 250 drop fractions collected in each tube. Based upon the UV-absorption (254 nm), thirteen fractions A2-M2 were collected. Among those, fractions E2 and G2 showed NF-1 activity and were further purified by HPLC. HPLC separation was performed on 53.0 mg of fraction E2 dissolved in 5.3 mL of MeOH and injected in 200 μ L aliquots (approx. 2.0 mg/injection) onto a 250 x 10 mm Varian Dynamax Microsorb 60-8 C₁₈ HPLC column. The detector wavelength was 225 nm and solvent flow rate was 4.0 mL/min. Elution began with 40% MeOH/H₂O, isocratic for 1 minute, then a linear gradient was programmed from 40% to 100% MeOH at 20 minutes, then the column was flushed with 100% MeOH for 5 minutes. Three major UV-absorbing peaks were collected and evaporated *in vacuo* to yield compound **9** (12.2 mg), compound **10** (2.4 mg) and compound **3** (0.8 mg) eluting at 7.2, 9.3 and 16.3 minutes, respectively. The same HPLC conditions were employed for fraction G2 (33.7 mg) to yield compound **11** (2.1 mg) eluting at 12.2 minutes.

Fraction D (420.0 mg) was chromatographed on a 2.5 x 40 cm Sephadex LH-20 column and eluted with CH₂Cl₂/MeOH (1:1, v/v), with 200 drop fractions collected in each tube to give seven fractions A3-G3. Among the seven fractions, fraction D3, E3 and F3 were found to have activity. HPLC purification was performed on 16.0 mg of fraction D3 dissolved in 1.6 mL of MeOH and injected in 200 μ L aliquots (approx. 2.0 mg/injection) onto the same C₁₈ HPLC column. (225 nm, 4.0 mL/min). Elution conditions were the same as above. Three major UV-absorbing peaks were collected and evaporated *in vacuo* to yield compounds **8** (0.4 mg), **1** (0.5 mg) and **2** (0.4 mg) eluting at 10.5, 13.2, and 18.1 minutes, respectively. Similar HPLC of fraction E3 (16.0 mg) yielded compounds **5** (1.3 mg) eluting at 13.2 minute, whereas, HPLC of fraction G3 (38.0 mg) yielded compounds **6** (1.0 mg) and **7** (1.1 mg) eluting at 9.3 and 9.5 minutes, respectively.

Canthin-6-one-9-methoxy-5-O- β -D-glucopyranoside (1)—Yellow amorphous powder; $[\alpha]_D^{20} = -48.2$ (c 0.2, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (4.02), 241 (4.23), 274 (4.11), 315 (3.89), 348 (4.01), and 365 (3.98) nm; IR (CHCl₃) $\nu_{\max}$ 3415, 2926, 1650, 1585, 1494, 1451, 1403 and 1050 cm⁻¹; ¹H (600 MHz, DMSO-d₆) and ¹³C (150 MHz, DMSO-d₆) NMR data, see Table 1; HREIMS [M+H]⁺ at *m/z* 429.1288 (calcd. for C₂₁H₂₁N₂O₈ 429.1298).

3.4 Bioassay

A previously described assay (Hawes et al., 2008) was used to assess the ability of samples to arrest growth and induce cytotoxicity in *Nf1*-null/*Trp53*-null murine astrocytoma cells. Briefly, *Nf1*^{-/-};*Trp53*^{-/-} KR158 astrocytoma cells expressing two luciferase constructs, one (resulting in green luminescence) driven by the human E2F1 promoter and the other (resulting in red luminescence) driven by the CMV promoter. Cells were plated at 1000 cell/well in white 384-well plates. After attachment (4 h), extracts, fractions, or pure compounds were added and red and green luminescence assessed using Promega ChromoGlo reagent 2 d later. Inhibition of the green luminescence signal (E2F1) reflects growth inhibition while inhibition of the red signal (CMV) indicates loss of cell viability (Hawes et al., 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A novel canthine glycoside was identified from *Simarouba berteriana*.
- Known quassinoids from this plant possessed the best cell growth inhibition against NF1-deficient cancer cells.
- A lignin was also identified as having modest activity, but was also toxic.

Table 1¹H and ¹³C NMR spectral data of compound **1**

C	δ_{H} (J in Hz)	δ_{C}	HMBC
1	8.07, <i>d</i> (5.0)	114.2	C-2, C-12, C-14, C-15
2	8.73, <i>d</i> (5.0)	146.0	C-1, C-14, C-16
4	7.59, <i>s</i>	114.9	C-5, C-6, C-15, C-16
5	-	151.7	-
6	-	154.7	-
8	8.08, <i>d</i> (2.2)	101.4	C-9, C-10, C-12, C-13
9	-	161.6	-
10	7.21, <i>dd</i> (8.5, 2.2)	113.0	C-8, C-12
11	8.27, <i>d</i> (8.5)	124.5	C-8, C-9, C-13, C-14
12	-	117.8	-
13	-	140.1	-
14	-	129.1	-
15	-	127.6	-
16	-	135.5	-
1'	5.21, <i>d</i> (7.5)	100.2	C-5, C-2'
2'	3.38, <i>m</i>	73.0	C-1', C-3'
3'	3.51, <i>m</i>	77.3	C2', C-4'
4'	3.21, <i>m</i>	69.5	C-3', C-5'
5'	3.34, <i>m</i>	76.6	C-4', C-6'
6'	3.49, 3.74, <i>dd</i> (10.8, 4.2)	60.6	C-5', C-4'
OMe	3.95, <i>s</i>	55.8	C-9

Table 2

Cell viability and inhibition of proliferation (%) in *Nf1*- and *p53*-defective mouse CNS tumor cell line by compounds **1–10** at 2.0 µg/mL.

Compounds	Cell viability (untreated controls = 100)	% Inhibition of proliferation
1	51	71
2	41	76
3	51	59
4	56	48
5	59	70
6	54	76
7	54	70
8	54	48
9	121	71
10	251	57
11	86	57