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Compounds Obtained from *Sida acuta* with the Potential to Induce Quinone Reductase and to Inhibit 7,12-Dimethylbenz-[*a*]anthracene-Induced Preneoplastic Lesions in a Mouse Mammary Organ Culture Model

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Activity-guided fractionation of the EtOAc-soluble extract of the whole plants of *Sida acuta* using a bioassay based on the induction of quinone reductase (QR) in cultured Hepa 1c1c7 mouse hepatoma cells, led to the isolation of ten active compounds of previously known structure, quindolinone (**1**), cryptolepinone (**2**), 11-methoxyquindoline (**3**), *N-trans*-feruloyltyramine (**4**), vomifoliol (**5**), loliolide (**6**), 4-ketopinoresinol (**7**), scopoletin (**8**), evofolin-A (**9**), and evofolin-B (**10**), along with five inactive compounds of known structure, ferulic acid, sinapic acid, syringic acid, (±)-syringaresinol, and vanillic acid. These isolates were identified by physical and spectral data measurement. A new derivative of quindolinone, 5,10-dimethylquindolin-11-one (**1a**) was synthesized and characterized spectroscopically. Of the active substances, compounds **1-3** and **1a** exhibited the most potent QR activity, with observed CD (concentration required to double induction) values ranging from 0.01 to 0.12 μg/mL. Six compounds were then evaluated in a mouse mammary organ culture assay, with cryptolepinone (**2**), *N-trans*-feruloyltyramine (**4**), and 5,10-dimethylquindolin-11-one (**1a**) found to exhibit 83.3, 75.0, and 66.7% inhibition of 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions, respectively, at a dose of 10 μg/mL.

Key words: *Sida acuta*, Malvaceae, Indoloquinoline alkaloids, Quinone reductase induction, Mouse mammary organ culture (MMOC) assay, Cancer chemoprevention

INTRODUCTION

Sida acuta Burm. f. (Malvaceae; common name "Pichana negra" or "Morning Mallow") is a pantropical herb of wide geographic distribution (Long and Lakela, 1971). In Central America, this plant is used for asthma, renal inflammation, colds, fever, headache, and ulcers, and as an anti-worm medication (Coee and Anderson, 1996; Caceres *et al.*, 1987). Previous phytochemical investigations on this plant have resulted in the isolation of several alkaloidal and steroidal constituents (Cao and Qi, 1993; Dinan *et al.*, 2001; Gunatilaka *et al.*, 1980; Rao *et al.*, 1984; Prakash *et al.*, 1981). In our ongoing project directed toward the discovery of novel naturally occurring cancer chemopreventive agents (Kinghorn *et al.*, 2003; Pezzuto *et al.*, 1999), the whole plants of *Sida acuta* were chosen for more detailed investigation, since the EtOAc-soluble fraction of a MeOH extract significantly induced the enzyme NADP(H):quinone

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oxidoreductase (QR) in cultured Hepa 1c1c7 (mouse hepatoma) cells.

Cancer chemoprevention involves the prevention, delay, or reversal of the process of carcinogenesis through ingestion of dietary or pharmaceutical agents (Hong and Sporn, 1997; Pezzuto *et al.*, 1999). A large number of potential chemopreventive agents are known, some of which have proven effective in clinical trials (Hong and Sporn, 1997; Kelloff *et al.*, 1992). These agents may function by a variety of mechanisms, directed at all major stages of carcinogenesis (Wattenberg, 1997). One mechanism of note involves the induction of phase II detoxification enzymes, such as QR and GST (glutathione *S*-transferase) (Talalay *et al.*, 1981). This type of response is associated with the metabolic detoxification of carcinogens (Wattenberg, 1997; Talalay *et al.*, 1981), but certain events associated with later stages of the carcinogenic process may also be inhibited (Maxuitenko *et al.*, 1993). Thus, induction of phase II enzymes is regarded as an important mechanism of cancer chemoprevention.

Bioassay-guided fractionation of an EtOAc-soluble residue of *Sida acuta* using the QR induction assay led to the isolation and characterization of ten active constituents of previously known structure (**1-10**), including the indoloquinoline alkaloids, quindolinone (**1**), cryptolepinone (**2**), and 11-methoxyquindoline (**3**), along with five inactive compounds of known structure. A new derivative, 5,10-dimethylquindolin-11-one (**1a**), was synthesized from quindolinone (**1**). Six compounds were then chosen for evaluation in a mouse mammary organ culture assay which was used as a secondary biological discriminator (Mehta and Moon, 1991).

MATERIALS AND METHODS

Plant material

The whole plants of *Sida acuta* Burm. f. were collected in Coronel Portillo Province, Peru in July, 1998 by two of us (J. S. V. and J. G. G.). A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. Graham and Schunke 524).

General experimental procedures

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and Bruker DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. LREIMS, LRCIMS, and HRCIMS were recorded on a Finnigan MAT 95 instrument operated at 70 eV and ESIMS on a Hewlett-Packard 5989B mass spectrometer with a 5998A electrospray interface. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich, Milwaukee, WI) followed by charring at 110°C for 5-10 min. Silica gel (Merck 60A, 70-230 or 200-400 mesh ASTM) and Sephadex LH-20 (Sigma) were used for column chromatography. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use.

Extraction and isolation

The dried and milled plant material (2.6 kg) was extracted with MeOH (3×11 L) by maceration. The extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract was suspended in 90% MeOH and then partitioned with petroleum ether (3×1.5 L) to afford a petroleum ether-soluble syrup (D001) on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (2 L) and partitioned again with EtOAc (3×1.5 L) to give an EtOAc-soluble extract (D002) and an aqueous residue (D003). The CD values of the solvent partitions, D001, D002, and D003, were 2.79, 0.43, and >10 μg/mL, respectively.

Based on the above activity results, the EtOAc-soluble extract (D002, 11 g) was chromatographed over silica gel as stationary phase using a $CHCl_3$-MeOH gradient (from 1:0 to 1:1 v/v) as mobile phase to afford 15 pooled fractions (F004-F018). Of these, fractions F007-F010 and F012-F014 (CD values of 4.4, 5.2, 7.3, 3.0, 3.4, 3.0, and 3.4 μg/mL, respectively) showed QR-inducing activity. Fractions F007-F010 [eluted with $CHCl_3$-MeOH (24:1 v/v); 1.1 g] were combined, and then passed over a Sephadex LH 20 column, with $CHCl_3$-MeOH (1:3) used as solvent system, to give, in sequence: evofolin-A (**9**, 2.3 mg, 0.00009%), oil, $[\alpha]_D^{25}$ -34.8° (*c* 0.14, $CHCl_3$) {lit. $[\alpha]_D$ -28.57° (*c* 0.021, $CHCl_3$) (Wu *et al.*, 1995)}; loliolide (**6**, 6.7 mg, 0.00026%), mp 148-149°C, $[\alpha]_D^{25}$ -67.8° (*c* 0.34, $CHCl_3$) {lit. mp 148.5-149°C, $[\alpha]_D^{20}$ -87° (*c* 0.66, $CHCl_3$) (Tanaka and Matsunaga, 1989)}; scopoletin (**8**, 0.9 mg, 0.00003%), mp 201-203°C [(lit. mp 203-204°C) (Kang *et al.*, 1998)]; 4-ketopinoresinol (**7**, 1.2 mg, 0.00005%), mp 78-80°C, $[\alpha]_D^{25}$ +48.0° (*c* 0.1, MeOH) {lit. $[\alpha]_D^{20}$ +54.7° (*c* 0.71, MeOH) (Otsuka *et al.*, 1989)}; (±)-syringaresinol (4.2 mg, 0.00016%), mp 174-175°C, $[\alpha]_D^{25}$ 0° (*c* 0.2, $CHCl_3$), [lit. mp 174°C, optically inactive in $CHCl_3$ (Nawwar *et al.*, 1982)]; vanillic acid (7 mg, 0.00027%), mp 210-212°C; and ferulic acid (1.2 mg, 0.00005%), mp 164-166°C, respectively.

Fractions F012 and F013 [eluted with $CHCl_3$-MeOH (19:1); 0.8 g] were combined and then chromatographed

over Sephadex LH-20, with $CHCl_3$-MeOH (1:3) used as solvent system, to produce subfractions F040-F048. Further chromatographic separation of the combined fractions (F043 and F044) was carried out by preparative TLC (*n*-hexane-Me_2CO-HOAc, 5:4.8:0.2 as developing solvent), to afford: evofolin-B (**10**, 1.2 mg, 0.00005%, R_f= 0.37), oil, $[\alpha]_D^{25}$ -11.2° (*c* 0.1, $CHCl_3$) {lit. $[\alpha]_D$ -14.3° (*c* 0.021, MeOH) (Wu *et al.*, 1995)}; sinapic acid (3.1 mg, 0.00012%, R_f=0.45), mp 200-202°C; and syringic acid (2.7 mg, 0.0001%, R_f=0.52), mp 187-188°C. Quindolinone (**1**, 11 mg, 0.00042%), mp >300°C and cryptolepinone (**2**, 3.9 mg, 0.00015%), mp >300°C [(lit. mp >300°C) (Fort *et al.*, 1998)] were obtained as bright yellow needles by recrystallization in MeOH from fractions F046 and F047, respectively.

The last active fraction, F014 [eluted with $CHCl_3$-MeOH (14:1 v/v); 1.7 g], was purified over a further Sephadex LH-20 column, with $CHCl_3$-MeOH (1:3 v/v) used as solvent system, yielding, in turn, the known compounds 11-methoxyquindoline (**3**, 8.2 mg, 0.00032%), mp 178-182°C, vomifoliol (**5**, 12.1 mg, 0.00047%), mp 110-112°C, $[\alpha]_5^{25}$ +191° (*c* 0.2, MeOH) {lit. mp 107-109°C, $[\alpha]_D$ +178.6° (*c* 1.65, $CHCl_3$) (Iida *et al.*, 1983)}, and *N-trans*-feruloyltyramine (**4**, 9.8 mg, 0.00038%), mp 92-94°C [(lit. mp 91°C) (Fukuda *et al.*, 1983)].

Methylation of quindolinone (1)

Compound **1** (9.0 mg), BaO (30 mg), and KOH (10 mg) were mixed in a 25 mL round-bottomed flask, and 5 mL acetone were then added in the flask, refluxed for 1 h. Excess iodomethane (50 µL) was added to the cooled residue and the mixture was refluxed for an additional 3 h. The solvent was removed under reduced pressure, and then the remaining mixture was partitioned between H_2O (5 mL) and $CHCl_3$ (5 mL) three times. The methylation product **1a** (8.5 mg) was obtained after evaporating the combined $CHCl_3$ solution.

5,10-Dimethylquindolin-11-one (1a)

Bright yellow powder: mp 191-192°C; IR ν_{max} NaCl cm^{-1}: 1619, 1587, 1519, 1466, 1377, 1281, 1165, 956, 743; UV λ_{max} EtOH nm (log ε): 251 (3.84), 315 (4.14), 327 (*sh*, 3.85), 383 (*sh*, 3.64), 396 (3.88); ^{1}H-NMR ($CDCl_3$, 360 MHz) δ 8.62 (1H, dd, *J* = 8.1 and 1.5 Hz, H-1), 8.16 (1H, d, *J* = 8.3 Hz, H-6), 7.63 (1H, dt, *J* = 7.0 and 1.6 Hz, H-3), 7.56 (1H, d, *J* = 8.1 Hz, H-4), 7.50 (1H, brt, *J* = 7.2 Hz, H-8), 7.41 (1H, d, *J* = 8.4 Hz, H-9), 7.29 (1H, brt, *J* = 7.2 Hz, H-2), 7.18 (1H, brt, *J* = 8.0 Hz, H-7), 4.35 (3H, s, 10-NCH_3), 4.26 (3H, s, 5-NCH_3); ^{13}C-NMR ($CDCl_3$, 90 MHz) δ 169.5 (C-11), 140.1 (C-4a and C-9a), 131.2 (C-3), 131.1 (C-5a), 127.3 (C-8), 126.6 (C-1), 124.9 (C-11a), 122.8 (C-10a), 122.6 (C-6), 120.9 (C-2), 119.2 (C-7), 115.7 (C-5b), 114.2 (C-4), 110.2 (C-9), 36.0 (5-NCH_3), 31.4 (10-NCH_3); CIMS *m/z* (rel. int.): 263 ($[M+H]^+$, 100), 249 (10), 233 (8), 75 (21); HRCIMS *m/z* 263.1188 ($[M+H]^+$, calcd for $C_{17}H_{15}N_2O$, 263.1184).

Quinone reductase induction assay with cultured mouse hepatoma cells

For the evaluation of plant extracts, fractions, and pure isolates as inducers of quinone reductase (QR), cultured mouse Hepa 1c1c7 cells (supplied by Dr. J. P. Whitlock, Jr., Stanford University, Stanford, CA) were used as described previously (Gerhäuser *et al.*, 1997; Jang *et al.*, 2002; Prochaska and Santamaria, 1988).

Mouse mammary organ culture assay

The inhibition of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced preneoplastic lesions in a mouse mammary organ culture model was performed using an established protocol (Jang *et al.*, 2002; Mehta and Moon, 1991).

RESULTS AND DISCUSSION

The known compounds, quindolinone (**1**) (Crouch *et al.*, 1995), cryptolepinone (**2**) (Fort *et al.*, 1998; Martin *et al.*, 1998), 11-methoxyquindoline (**3**) (Görlitzer and Ventzke-Neu, 1997), *N-trans*-feruloyltyramine (**4**) (Fukuda *et al.*, 1983; Hussain *et al.*, 1982), vomifoliol (**5**) (Iida *et al.*, 1983), loliolide (**6**) (Tanaka and Matsunaga, 1989), 4-ketopinoresinol (**7**) (Otsuka *et al.*, 1989), scopoletin (**8**) (Kang *et al.*, 1998), evofolin-A (**9**), evofolin-B (**10**), ferulic acid (Han *et al.*, 1983), sinapic acid (Wettasinghe *et al.*, 2001), syringic acid (Wettasinghe *et al.*, 2001), (±)-syringaresinol (Nawwar *et al.*, 1982), and vanillic acid (Huang *et al.*, 1993) were isolated from an EtOAc-soluble fraction of *Sida acuta* by bioassay-guided fractionation using the QR induction assay. Their structures were identified by physical and spectroscopic data (mp, $[\alpha]_D$, MS, ^{1}H- and ^{13}C-NMR) measurement and by comparison with published values. A new derivative, 5,10-dimethylquindolin-11-one (**1a**), was synthesized from quindolinone (**1**) to compare its biological activity with those of **1** and **2**. Compounds **1-7**, **9**, **10**, and (±)-syringaresinol have not been isolated from any species in the genus *Sida* previously.

The potential of compounds **1-10** and **1a** to induce QR activity in Hepa1c1c7 cells is summarized in Table I. In laboratory animals and cell culture systems, several chemopreventive agents have been identified by others on the basis of their ability to induce phase II enzymes (Lam *et al.*, 1982; Zhang *et al.*, 1992). Similarly, in our program directed toward the discovery of novel chemopreventive agents, we have used cultured Hepa 1c1c7 cells to evaluate the induction of QR activity, which has led to the identification of various active agents, including diarylheptanoids (Jang *et al.*, 2002), flavonoids (Chang *et al.*, 1997; Gu *et al.*, 2002), and withanolides (Kennelly *et*

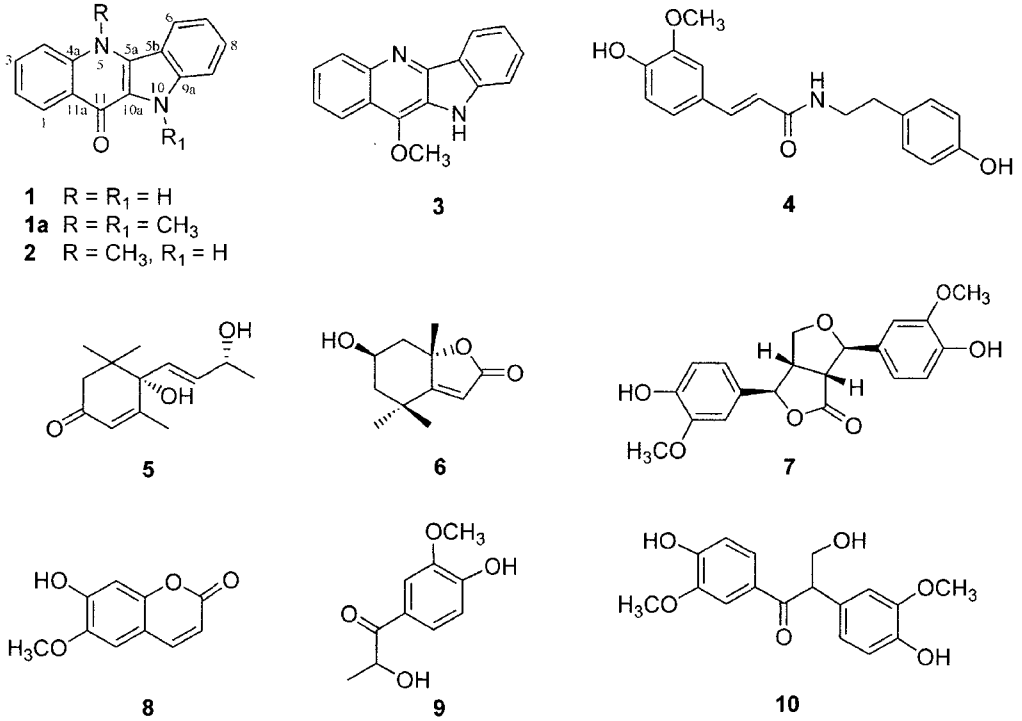

Fig. 1. Structures of compounds **1-10** isolated from *S. acuta*

al., 1997; Su *et al.*, 2002; Su *et al.*, 2003). In the present work, the indoloquinoline alkaloids **1-3** exhibited potent QR activity, with the observed concentration to double induction (CD) values ranging from 0.01 to 0.12 μg/mL. Furthermore, superior chemopreventive index (CI; >62.5) values were observed for these compounds as a result of their limited cytotoxicity (Table I). The QR-inducing potency of quindolinone (**1**; CD value; 0.12 μg/mL) was increased significantly by methylation at position N-5 as in cryptolepinone (**2**; CD value; 0.02 μg/mL), but the di-*N*-methyl derivative, 5,10-dimethylquindolin-11-one (**1a**; CD value; 0.6 μg/mL), showed reduced activity when compared with **1**. In turn, *N-trans*-feruloyltyramine (**4**), vomifoliol (**5**), loliolide (**6**), 4-ketopinoresinol (**7**), scopoletin (**8**), evofolin-A (**9**), and evofolin-B (**10**) induced QR activity, with CD values ranging from 1.6 to 8.5 μg/mL.

Finally, six selected compounds (**1-5**, and **1a**) were evaluated for their potential to inhibit DMBA-induced preneoplastic lesions in mouse mammary glands in organ culture (MMOC). As noted previously (Mehta and Moon, 1991), compounds active in this model system are considered good candidates for full-term cancer chemopreventive studies. As shown in Table I, cryptolepinone (**2**) exhibited the most significant response in the MMOC assay (83.3% inhibition at 10 μg/mL). Also, *N-trans*-feruloyltyramine (**4**) and 5,10-dimethylquindolin-11-one (**1a**) exhibited significant responses in this assay (75.0 and 66.7% inhibition at 10 μg/mL, respectively). Therefore,

Table I. Activity of compounds **1-10** from *S. acuta* in the quinone reductase (QR) induction and mouse mammary organ culture (MMOC) bioassays

Compound	QR[a]			MMOC (%)[b]
	CD (μg/mL)	IC_{50} (μg/mL)	CI	
1	0.12	>20	>167	28.5
1a	0.6	7.9	13.1	66.7
2	0.02	>5	>250	83.3
3	0.01	2.5	250	22.0
4	8.5	>20	>2.3	75.0
5	6.8	>20	>2.9	37.0
6	6.3	>20	>3.2	ND[c]
7	1.6	>20	>12.5	ND
8	3.9	>20	>5.1	ND
9	6.1	>20	>3.3	ND
10	5.2	>5	>0.9	ND
sulforaphane[d]	0.09	2.1	23.3	83.7

[a]CD, concentration required to double QR activity; IC_{50}, concentration inhibiting cell growth by 50%; CI, Chemoprevention Index (=IC_{50}/CD). Compounds with CD values of <10 μg/mL were considered active. [b]Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Selected compounds from *S. acuta* were tested at concentrations of 10 μg/mL. On the basis of historical controls, inhibition of >60% (at 10 μg/mL) is considered significant. [c]Not determined since the amount of available compound was insufficient. [d]Sulforaphane was used as a positive control, and was tested at a concentration of 1 μg/mL (Gerhäuser *et al.*, 1997).

cryptolepinone (**2**) is worthy of consideration as a potential cancer chemopreventive agent through additional biological evaluation. This compound was obtained originally as an oxidized extraction artifact of cryptolepine from *Cryptolepis sanguinolenta* (Lindl.) Schlecter (Asclepiadaceae) (Fort *et al.*, 1998), but no account of its biological activity appears has been published to date. In terms of its potential cancer chemopreventive activity, *N-trans*-feruloyltyramine (**4**) has been shown to exhibit antioxidant activity against lipid peroxidation in rat liver microsomes (Lee *et al.*, 1999).

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