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A sugar ester and an iridoid glycoside from *Scrophularia ningpoensis*

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Abstract

From cytotoxic extracts of the roots of *Scrophularia ningpoensis* Hemsl. (Scrophulariaceae) a new sugar ester, ningposide D (3-*O*-acetyl-2-*O*-*p*-methoxycinnamoyl-α(β)-L-rhamnopyranose) (**1**) and a new iridoid glycoside, scrophuloside B_4 (6-*O*-(2″-*O*-acetyl-3″-*O*-cinnamoyl-4″-*O*-*p*-methoxycinnamoyl-α-L-rhamnopyranosyl) catalpol) (**2**) along with known compounds: oleanonic acid (**3**), ursolonic acid (**4**), cinnamic acid (**5**), 3-hydroxy-4-methoxy benzoic acid (**6**), 5-(hydroxymethyl)-2-furfural (**7**) and β-sitosterol (**8**) were isolated. The structures of the new compounds were elucidated by spectral data (1, 2D NMR, EI, HRESI-MS and MS/MS). Oleanonic acid (**3**) and ursolonic acid (**4**) were found to be cytotoxic against a series of human cancer cell lines with IC_{50} = 4.6, 15.5 μM on MCF7; 4.2, 14.5 μM on K562; 14.8, 44.4 μM on Bowes; 24.9, 43.6 μM on T24S; 61.3, 151.5 μM on A549, respectively. β-Sitosterol (**8**) inhibited Bowes cells growth at IC_{50} = 36.5 μM. Scrophuloside B_4 (**2**) showed activity on K562 and Bowes cells at IC_{50} = 44.6, 90.2 μM, respectively.
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Keywords: *Scrophularia ningpoensis*; Sugar ester; Iridoid glycoside; Ningposide D; Scrophuloside B_4; Cytotoxicity

1. Introduction

The Scrophulariaceae family consists of 220 genera in which the genus *Scrophularia* is known for the rich presence of sugar esters and iridoid glycosides (Boros and Stermitz, 1990; Miyase and Mimatsu, 1999). *Scrophularia ningpoensis* Hemsl. is commonly known as ''Huyen sam'', ''Hac sam'' and ''Nguyen sam'' in Vietnam. Its dried roots are used as antipyretic, febrifuge and antibacterial, as a remedy for evening fever, erythema, mouth dryness, constipation, prurigo, furunculosis, sore throat, ulcerous stomatitis, tonsillitis and in the treatment of cancer (WHO and IMM, 1990; Nguyen et al., 2005). In previous studies, 11 sugar esters: ningposides A, B and C, sibirioside, cistanoside D, angoroside C, acteoside, decaffeoylacteoside, cistanoside F, 4-*O*-(*p*-methoxycinnamoyl)-α-L-rhamnopyranoside, 2-(3-hydroxy-4-methoxyphenyl)ethyl-1-*O*-[α-L-arabinopyranosyl (1–6)]-feruloyl (1–4)]-α-L-rhamnopyranosyl (1–3)-β-D-glucopyranoside and 13 iridoid glycosides: harpagoside, harpagide, aucubin, 6-*O*-methylcatalpol, scropoliside A, ningpogosides A and B, 8-*O*-(2-hydroxycinnamoyl) harpagide, 8-*O*-feruloyl harpagide, 6-*O*-α-D-galactopyranosyl harpagoside, geniposide, ningpogenin and 6′-*O*-acetyl harpagoside were isolated from the plant roots (Li et al., 1999, 2000; Kajimoto et al., 1989; Zhang et al., 1994; Lin et al., 1996; Qian et al., 1992; Zou and Yang, 2000). However, to our knowledge, these compounds have not been previously investigated for

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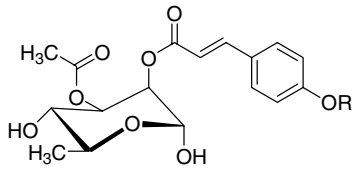
biological activities. In the present study, we report the isolation, structure determination and cytotoxicity against a series of human cancer cell lines of a new sugar ester and a new iridoid glycoside (**1**–**2**), and of six known compounds from the roots of *S. ningpoensis*.

2. Results and discussion

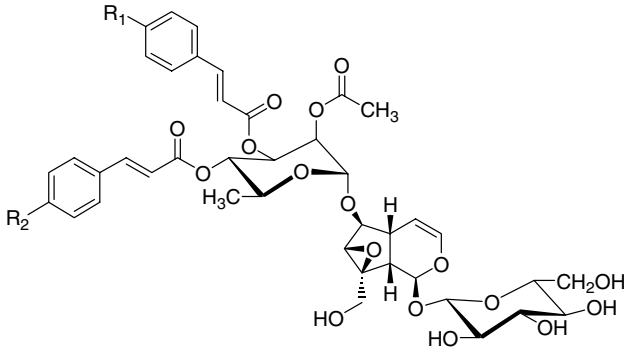
The CH_2Cl_2 and EtOAc extracts of *S. ningpoensis* powdered roots were fractionated and purified by silica gel, ODS CC and *prep.* TLC to yield a new sugar ester and a new iridoid glycoside that were named ningposide D (**1**) and scrophuloside B_4 (**2**), along with six known compounds: oleanonic acid (**3**) $[\alpha]_D^{25} + 76.9°$ (*c* 0.06, $CHCl_3$), ursolonic acid (**4**) $[\alpha]_D^{25} + 82.8°$ (*c* 0.34, $CHCl_3$), cinnamic acid (**5**), 3-hydroxy-4-methoxy benzoic acid (**6**), 5-(hydroxymethyl)-2-furfural (**7**) and β-sitosterol (**8**) $[\alpha]_D^{25} - 37°$ (*c* 0.5, $CHCl_3$). The structures of the known compounds (**3**–**8**) were identified by 1, 2 D-NMR, EIMS and by comparison with published data (Seo et al., 1975; Wright et al., 1978; Budavari et al., 1996).

Compound **1** was obtained as an oil, $[\alpha]_D^{25} + 42.0°$ (*c* 0.2, $CHCl_3$). The molecular formula $C_{18}H_{22}O_8$ was determined by EI-MS, *m/z* M^+ 366, ^1H, ^{13}C, DEPT 90 and DEPT 135-NMR. The ^1H NMR spectrum showed signals of an anomeric proton (δ_H 5.22, *d*, $J = 1.8$ Hz) and a secondary methyl group (δ_H 1.40, *d*, $J = 6.19$ Hz), indicating the presence of an α-L-rhamnose moiety (Agrawal, 1992). From the anomeric proton, we assigned every proton and carbon of the rhamnosyl group by detailed analysis of ^1H–^1H COSY, HMQC and HMBC spectra. In addition to these signals, the ^1H NMR spectrum of **1** exhibited signals of a cinnamoyl moiety: four aromatic protons (δ_H 7.49 *d* and 6.91 *d*, ABA′B′ system, $J = 8.8$ Hz, each 2 H) and two *trans* olefinic protons (δ_H 6.40 *d* and 7.66 *d*, AB system, $J = 16.0$ Hz). The ^1H and ^{13}C NMR data of **1** (Table 1) were similar to those of ningposide C (**9**) (Li et al., 2000), except for the signal of the methoxyl group at $\delta_H = 3.85$ (3H, *s*), $\delta_C = 55.4$. In the HMBC spectrum, the methoxyl protons gave cross-peak with the C-4-cinnamoyl at $\delta_C = 162.0$, indicating a methoxyl substitution at this position. In the same experiment, the sugar proton at $\delta_H = 5.40$ (*dd*, $J = 1.8$, 3.3 Hz, H-2) correlated with the carbonyl carbon at $\delta_C = 166.4$ of the cinnamoyl moiety, whereas other sugar proton at $\delta_H = 5.25$ (*dd*, $J = 3.3$, 9.8 Hz, H-3) gave cross-peak with another carbonyl carbon at $\delta_C = 171.3$, which was further correlated with the methyl protons at $\delta_H = 2.08$ (3H, *s*). Thus, compound **1** was identified as 3-*O*-acetyl-2-*O*-*p*-methoxycinnamoyl-α-L-rhamnopyranose that we have named ningposide D. In the ^1H NMR spectrum, we also observed 14 parallel smaller signals, which were assigned to 3-*O*-acetyl-2-*O*-*p*-methoxycinnamoyl-β-L-rhamnopyranose (the anomeric ration $\alpha/\beta \sim (3:1)$). The base peak at *m/z* 161 in the EI mass spectrum corresponds to the methoxycinnamoyl cation $[C_{10}H_9O_2]^+$. The other abundant ion at *m/z* 133 is due to subsequent loss of CO, while the abundant ion at *m/z* 178 is explained by loss of a rhamnose residue (162 u) from the M^+.



(**1**) Ningposide D: R = CH_3

(**9**) Ningposide C: R = H



(**2**) Scrophuloside B_4: R_1 = H, R_2 = OCH_3

(**10**) Scrophuloside A_4: R_1 = OCH_3, R_2 = OCH_3

(**11**) Scropolioside B: R_1 = H, R_2 = H

Compound **2** was obtained as a yellowish powder, $[\alpha]_D^{25} - 31.8°$ (*c* 0.29, $CHCl_3$). The molecular formula $C_{42}H_{48}O_{18}$ was determined by positive HRESI-MS, $[M + Na]^+$ ion (*m/z* 863.2744; Calc. 863.2738) and negative HRESI-MS, $[M - H]^-$ ion (*m/z* 839.2747; Calc. 839.2762), ^1H-, ^{13}C-, DEPT 90 and DEPT 135-NMR. The UV spectrum of **2** exhibited absorption bands that are characteristic of an iridoid enol ether system and cinnamoyl chromophores (λ_{max} at 216, 222 and 282 nm) (Calis et al., 1993). The ^1H NMR spectrum showed signals of two anomeric protons (δ_H 4.82, *d*, $J = 7.8$ Hz; δ_H 5.03, *d*, $J = 1.2$ Hz) and the secondary methyl group (δ_H 1.28, *d*, $J = 6.6$ Hz), indicating the presence of two sugar moieties (α-L-rhamnose and β-D-glucose) (Agrawal, 1992). The spectroscopic data of **2** (Table 1) are quite similar to those of scrophuloside A_4 (**10**) (Miyase and Mimatsu, 1999) and scropolioside B (**11**) (Calis et al., 1988), except for the signals of the aromatic protons, which suggested the presence of both a *p*-methoxycinnamoyl group and an unsubstituted cinnamoyl group. This was supported by a HMBC experiment that showed long range correlation of the methoxyl protons at δ_H

Table 1
^{1}H and ^{13}C NMR spectroscopic data of ningposide D (**1**) and scrophuloside B_4 (**2**) in $CDCl_3$ and in MeOD

Assignments		Ningposide D (**1**) in $CDCl_3$			Scrophuloside B_4 (**2**) in $CDCl_3$ and MeOD	
		δ_C (ppm)	δ_H (ppm) *J* (Hz)		δ_C (ppm)	δ_H (ppm) *J* (Hz)
Rhamnosyl	1	92.5	5.22 *d* (1.8)	1″	96.6	5.03 *d* (1.2)
	2	70.2	5.40 *dd* (1.8, 3.3)	2″	70.4	5.41 *dd* (1.2, 3.0)
	3	72.1	5.25 *dd* (3.3, 9.8)	3″	69.1	5.53 *dd* (3.0, 9.6)
	4	71.5	3.75 *t* (9.8)	4″	71.7	5.34 *t* (9.6)
	5	68.7	4.05 *m*	5″	67.3	4.10 *m*
	6	17.7	1.40 *d* (6.1, 3H)	6″	17.5	1.28 *d* (6.6, 3H)
Acetyl	1	171.3	–	1	170.5	–
	2	21.0	2.08 *s* (3H)	2	21.0	2.20 *s* (3H)
Cinnamoyl				1	134.1	–
				2	128.9	7.47 *dd* (2.5, 8.5)
				3	129.0	7.36 *dd* (8.5, 8.5)
				4	130.6	7.35 *dd* (8.5, 8.5)
				5	128.9	7.36 *dd* (8.5, 8.5)
				6	128.3	7.49 *dd* (8.5, 2.5)
				α	117.0	6.33 *d* (16.0)
				β	146.2	7.63 *d* (16.0)
				COO	166.1	–
p-Methoxycinnamoyl	1	128.0	–	1	126.8	–
	2	130.1	7.49 *d* (8.8)	2	130.2	7.46 *d* (9.0)
	3	114.4	6.91 *d* (8.8)	3	114.4	6.89 *d* (9.0)
	4	162.0	–	4	161.7	–
	5	114.4	6.91 *d* (8.8)	5	114.4	6.89 *d* (9.0)
	6	130.1	7.49 *d* (8.8)	6	130.2	7.46 *d* (9.0)
	α	114.4	6.40 *d* (16.0)	α	114.3	6.25 *d* (16.2)
	β	145.7	7.66 *d* (16.0)	β	146.1	7.64 *d* (16.2)
	OCH_3	55.4	3.85 *s* (3H)	OCH_3	55.5	3.83 *s* (3H)
	COO	166.4	–	COO	166.9	–
Glucosyl				1′	99.0	4.82 *d* (7.8)
				2′	73.2	3.36 *dd* (7.8, 7.2)
				3′	76.2	3.51 *dd* (7.2, 7.8)
				4′	69.6	3.49 *dd* (7.8, 7.2)
				5′	76.7	3.38 *m*
				6′	61.4	*a*. 3.88 *dd* (11.4, 5.5)
						b. 3.77 *dd* (2.0, 11.4)
Aglycone				1	94.6	4.92 *d* (9.6)
				2	–	–
				3	141.3	6.36 *d* (5.8)
				4	102.5	5.13 *dd* (5.8, 4.7)
				5	35.9	2.55 *m*
				6	83.6	4.02 *dd* (8.7, 2.1)
				7	58.4	3.65 *d* (2.1)
				8	65.0	–
				9	42.4	2.66 *dd* (9.6, 7.8)
				10	60.9	*a.* 3.98 *dd* (11.0, 2.0)
						b. 4.00 *dd* (5.5, 11.0)

3.83 (3H, *s*) to C-4 of the *p*-methoxycinnamoyl moiety. The assignments of protons and carbons of **2** were made by detailed analysis of heteronuclear multiple quantum coherence HMQC, ^{1}H–^{1}H correlation spectroscopy COSY and long range ^{1}H–^{13}C heteronuclear correlation HMBC spectra. Starting from the two easily distinguishable carbonyl carbons at δ_c 166.1 and δ_c 166.9 we assigned every proton and carbon of the cinnamoyl and *p*-methoxycinnamoyl moieties; then from the acetal methine proton H-1 (δ_H 4.92, *d*, *J* = 9.6 Hz) we assigned every proton and carbon of the iridoid enol ether ring; and from the two anomeric protons we assigned every proton and carbon of the two sugar units. In the HMBC spectrum, H-1 of the iridoid enol ether ring gave cross-peak with C-1′ of the glucose whereas H-1′ of the glucose gave cross-peak with C-1 of the iridoid enol ether ring. These elements indicated that the etherification was between position 1 of the iridoid enol ether ring and position 1′ of the glucose unit. In the same experiment, H-1″ of the rhamnose gave cross-peak with C-6 of the iridoid enol ether ring whereas H-6 of the iridoid enol ether ring gave cross-peak with C-1″ of the

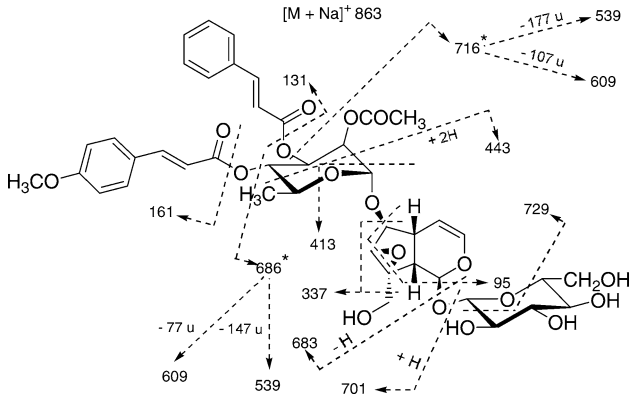


Fig. 1. Product ions detected in the high-energy $[M + Na]^+$ CID spectrum of **2**.

rhamnose. The ether linkage was then between position 6 of the iridoid enol ether ring and position 1″ of the rhamnose unit. The HMBC experiment also showed that H-2″ of the rhamnose correlated with the carbonyl carbon at $\delta_C = 170.5$, which was further correlated with the methyl protons at $\delta_H = 2.20$ (3H, *s*); H-3″ and H-4″ of the rhamnose correlated with the carbonyl carbons of the cinnamoyl and the *p*-methoxycinnamoyl moieties, respectively. The results then indicated that the acetyl group was located at C-2″, the cinnamoyl group at C-3″, and the *p*-methoxycinnamoyl moiety at C-4″ of the rhamnose. The structure of **2** therefore was elucidated to be 6-*O*-(2″-*O*-acetyl-3″-*O*-cinnamoyl-4″-*O*-*p*-methoxycinnamoyl-α-L-rhamnopyranosyl) catalpol, a new natural compound for which the name scrophuloside B_4 is now proposed. The similarities of ^{13}C NMR assignments of a compound isolated from the same plant by Kajimoto et al. (1989) to those of our compound suggested that it could be likened to compound **2**. However, available spectroscopic data did not allow to assign a complete structure; some of these NMR data are here revised, i.e., the assignments of aromatic carbons of the cinnamoyl moiety.

The structure of scrophuloside B_4 (**2**) was also supported by ESI-MS in combination with collision-induced dissociation (CID) and tandem mass spectrometry (ES-MS/CID/MS). The high-energy $[M + Na]^+$ CID data are summarized in Fig. 1. The three most abundant product ions were at *m*/*z* 161 (methoxycinnamoyl cation), *m*/*z* 131 (cinnamoyl cation) and *m*/*z* 95 (due to fragmentation in the catalpol part). Structurally informative radical ions (indicated by an asterisk in Fig. 1) were present at *m*/*z* 716 and *m*/*z* 686, due to loss of a cinnamoyloxy and a methoxycinnamoyloxy radical, respectively. The ions at *m*/*z* 683 (loss of 180 u), *m*/*z* 701 (loss of 162 u) and *m*/*z* 729 (loss of 134) are consistent with a terminal glucose residue. In addition, several other ions were observed (Fig. 1), which could all be rationalized on the basis of the proposed structure.

The isolated compounds (**1**–**8**) were tested for cytotoxicity against a series of human cancer cell lines, MCF7, K562, Bowes, T24S and A549. As shown in Table 2, oleanonic acid (**3**) and ursolonic acid (**4**) were found to be the most active compounds with IC_{50} values ranging between 4.0 and 151.5 μM, the activity being somewhat lower on A549 cells. The olean-12-ene derivative (**3**), however, was more active than the urs-12-ene derivative (**4**) on all tested cells. β-Sitosterol (**8**) inhibited Bowes cells growth at $IC_{50} = 36.5$ μM. Scrophuloside B_4 (**2**) showed cytotoxic activity on K562 and Bowes but not on other cell lines. All other compounds were considered inactive.

3. Experimental

3.1. General

TLC was carried out on precoated silica gel 60 F254 plates (Merck). Spots were detected under UV (254 and 366 nm) before and after spraying with an anisaldehyde

Table 2
Cytotoxicity[a] of **1**–**8** (MTT cytotoxicity assay, data from three independent experiments, each in hexaplicate; 72 h incubation)

Compound	IC_{50} (μM) cell lines				
	MCF7	K562	Bowes	T24S	A549
1	>100	>100	>100	>100	>100
2	>100	44.6 ± 6.4	90.2 ± 7.7	>100	>100
3	4.6 ± 0.1	4.2 ± 0.3	14.8 ± 0.5	24.9 ± 0.5	61.3 ± 1.2
4	15.5 ± 1.1	14.5 ± 1.3	44.4 ± 2.2	43.6 ± 2.2	151.5 ± 0.1
5	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100
8	>100	>100	36.5 ± 3.8	>100	>100
Adriamycin[b]	1.5 ± 0.2	0.07 ± 0.01	0.45 ± 0.01	5.8 ± 0.6	15.8 ± 6.7

[a] The $IC_{50} \pm SD$ were determined by fitting experimental points to a parametric function by means of an original simplex algorithm: $N = N° \exp(-kC)$, where C is the concentration, N the percentage of living cells at concentration C, $N°$ the percentage of living cells at concentration 0 and k is the parameter (Khalil et al., 1986; Dubois et al., 1989).

[b] Cytotoxic reference compound.

sulfuric acid solution followed by heating the plate at 150 °C for 10 min. Prep. TLC was performed on precoated silica gel plates, layer thickness 0.25 mm (Merck). Column chromatography was carried out on silica gel 60 (230–400 mesh, *i.d.* 2 × 30 cm, Merck). ^{1}H and ^{13}C (BBD, DEPT 135, DEPT 90) NMR spectra were measured on a Bruker Avance 300 at 300 and 75 MHz, respectively, with TMS as an internal standard; 2-D NMR spectra including COSY, HMQC and HMBC were recorded in $CDCl_3$ and MeOD on a Varian Unity 600 at 25 °C. HRESI-MS were performed on a Micromass QTOF II Mass Spectrometer at a capillary and cone voltages of 2.8 kV and 80 V, respectively, and at a mass resolution of approximately 10,000. EI mass spectra were recorded on an Autospec M instrument (Micromass, Manchester, UK) at an ion source temperature of 200 °C, an electron energy of 70 eV and a mass resolution of approximately 500. ESI-MS and MS-MS were performed on an Autospec-oa-ToF mass spectrometer (Micromass) at a mass resolution of approximately 1500. High-energy CID spectra were acquired at a collision energy (E_{lab}) of 400 eV using xenon as collision gas. The collision gas was introduced into the collision cell until the $[M + Na]^+$ signal reached 60% of its original value. The optical rotations were recorded on a Perkin–Elmer 141 polarimeter at 25 °C. UV spectra were measured on a Shimadzu UV–Vis spectrometer UV-265FS.

3.2. Plant material

The *S. ningpoensis* roots were collected in Lang son, Vietnam in September 2002. The voucher specimen (N° 507) is deposited in the Herbarium of Hanoi University of Pharmacy, Vietnam.

3.3. Extraction and isolation

The air-dried roots (700 g) were macerated (24 h, room temperature) and exhaustively percolated with CH_2Cl_2 then with EtOAc. The CH_2Cl_2 and EtOAc extracts were concentrated under vacuum to dryness to yield 6.8 and 9.3 g, respectively. A mass of each extract was dissolved in DMSO and then diluted in cell growth culture medium for the MTT cytotoxicity assay (max conc. of DMSO in the test solution was 0.5%). Both CH_2Cl_2 and EtOAc extracts showed interesting cytotoxicity against several human cancer cell lines (Nguyen et al., 2005). 4.1 g of the EtOAc extract were submitted to silica gel CC and eluted with petrol (40–60 °C)-EtOAc (9:1; 6:1; 4:1; 2:1; 0:1), followed by EtOAc–MeOH (9:1; 6:1; 4:1; 2:1; 0:1), to yield 10 fractions (I–X). Fraction II was purified by crystallisation in cold EtOH to give compound **8** (8.5 mg). Fraction III was purified by prep. TLC using toluene–EtOAc–HOAc (40:10:5; two developments) to afford compound **6** (3 mg). Fraction V was first applied on prep. TLC using $C_2H_4Cl_2$–MeOH–EtOH–H_2O (60:10:10:1) and then $C_2H_4Cl_2$–EtOH (8:1), finally applied to ODS silica gel CC and eluted with MeOH–H_2O (1:1), to yield compound **1** (4 mg). Fraction VI was purified by prep. TLC using $CHCl_3$–MeOH (9:1) to yield compound **5** (10 mg).

6.0 g of the CH_2Cl_2 extract were applied to silica gel CC and eluted with $CHCl_3$–MeOH (9:1; 6:1; 4:1; 2:1; 0:1) to yield five fractions (A–E). Two of these fractions (B and C) were found to be active on five cell lines, MCF7, K562, Bowes, T24S and A549. Fraction B was then fractionated on silica gel CC using a gradient elution $CHCl_3$–MeOH (1:0; 9:1; 8:2) to yield six subfractions (B1–B6). Subfraction B2 was further fractionated on silica gel CC using a stepwise gradient mixture of $CHCl_3$–MeOH (50:1; 50:2; 50:3; 50:5) and on prep. TLC using petrol (40–60 °C)–Et_2O–HOAc (90:10:1; three developments) to yield compounds **3** (3 mg) and **4** (7 mg). Fraction C was submitted to silica gel CC using a stepwise gradient mixture of C_6H_{12}–Me_2CO (9:1; 7:3; 5:8; 3:7; 2:8; 1:9), followed by C_6H_{12}–Me_2CO–MeOH (1:9:0.1; 1:9:0.2; 1:9:0.3; 1:9:1). Subfractions were further fractionated on silica gel CC using a stepwise gradient mixture of petrol (40–60 °C)–Et_2O–HOAc (90:10:1; 80:10:2; 50:50:5) then by prep. TLC using petrol (40–60 °C)–Et_2O–HOAc (50:50:5) or $CHCl_3$–MeOH (9:1) to afford compounds **7** (11 mg) and **2** (7 mg).

3.4. Cytotoxicity testing

A549 cells (non-small cell lung cancer) and MCF7 cells (breast cancer) were obtained from ATCC, Manassas, USA; Bowes cells (skin cancer) and T24S cells (bladder cancer) were a generous gift from Professor Mareel, Lab. of Experimental Cancerology, UZ Gent, Belgium; K562 cells (leukemia) was received from Professor Zizi, Lab. of Neurophysiology, VUB, Belgium. MTT cytotoxicity assay was carried out according to a procedure previously described; the cells were incubated with drug solutions for 72 h (Nguyen et al., 2004; Camby et al., 1996).

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References

Agrawal, P.K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307–3330.

Boros, C.A., Stermitz, F.R., 1990. Iridoids. An updated review. Part I. J. Nat. Prod. 53, 1055–1147.

Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P.E., Kinneary, J.F., 1996. β-Sitosterol, 12th ed.The Merck Index Merck & Co. Inc., London, p. 1467.

Calis, I., Gross, A.G., Winkler, T., Sticher, O., 1988. Isolation and structure elucidation of two highly acetylated iridoid diglycosides from *Scrophularia scopolii*. Planta Med. 54, 168–170.

Calis, I., Zor, M., Basaran, A.A., 1993. Karsoside and scropolioside D, two new iridoid glycosides from *Scrophularia ilwensis*. J. Nat. Prod. 56, 606–609.

Camby, I., Salmon, I., Danguy, A., Pasteels, J.L., Brotchi, J., Martinez, J., Kiss, R., 1996. Influence of gastrin on human astrocytic tumour cell proliferation. J. Natl. Cancer Inst. 88, 594–600.

Dubois, J., Khalil, A.F., Hanocq, M., Atassi, G., Arnould, R., 1989. Ajustement des courbes dose-effet au moyen d'un algorithme original. Application à des études de cytotoxicité in vitro. J. Pharm. Belg. 44, 181–191.

Kajimoto, T., Hidaka, M., Shoyama, K., Nohara, T., 1989. Iridoids from *Scrophularia ningpoesis*. Phytochemistry 28, 2701–2704.

Khalil, A.F., Dubois, J., Hanocq, M., Atassi, G., 1986. A new algorithm for computing the parameters of linear compartment models in pharmacokinetics. Eur. J. Drug Metab. Pharmacokinet. 1, 51–59.

Li, Y.M., Jiang, S.H., Gao, W.Y., Zhu, D.Y., 1999. Iridoid glycosides from *Scrophularia ningpoensis*. Phytochemistry 50, 101–104.

Li, Y.M., Jiang, S.H., Gao, W.Y., Zhu, D.Y., 2000. Phenylpropanoid glycosides from *Scrophularia ningpoensis*. Phytochemistry 54, 923–925.

Lin, J.H., Ku, Y.R., Huang, Y.S., Yarn, F.M., Wen, K.C., 1996. Isolation and determination by HPLC polar constituents from *Scrophularia ningpoensis*. Yaowu Shipin Fenxi 4, 131–139.

Miyase, T., Mimatsu, A., 1999. Acetylated iridoid and phenylethanoid glycosides from the aerial parts of *Scrophularia nodosa*. J. Nat. Prod. 62, 1079–1084.

Nguyen, A.T., Fontaine, J., Malonne, H., Vanhaelen, M., Duez, P., 2005. Cytotoxicity of five plants used as anticancer remedies in Vietnam traditional medicine. Ethnopharmacol, submitted.

Nguyen, A.T., Malonne, H., Duez, P., Vanhaelen, M., Fontaine, J., 2004. Cytotoxic constituents from *Plumbago zeyl anica*. Fitoterapia 75, 500–504.

Qian, J., Hunkler, D., Rimpler, H., 1992. Iridoid-related aglycone and its glycosides from *Scrophularia ningpoensis*. Phytochemistry 31, 905–911.

Seo, S., Tomita, Y., Tri, K., 1975. Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus* Hara tissue cultures. Tetrahedron Lett. 16, 7–10.

World Health Organisation (WHO) and Institute of Materia Medica (IMM), 1990. Medicinal Plants in Vietnam. Scientific and Technical Publishing House, Hanoi, pp. 342–343.

Wright, J.L.C., Mcinnes, A.G., Shimizu, S., Smith, D.G., Walter, J.A., Idler, D., Khalil, W., 1978. Identification of C-24 alkyl epimers of marine sterols by ^{13}C nuclear magnetic resonance spectroscopy. Can. J. Chem. 56, 1898–1903.

Zhang, W., Liu, Y., Li, X., Pu, X., Jin, Y., Yang, C., 1994. Chemical constituents from *Scrophularia ningpoensis*. Yunnan Zhiwu Yanjiu 16, 407–412.

Zou, C., Yang, X., 2000. A new iridoid glucoside from ningpo fig-wort (*Scrophularia ningpoensis*). Zhongcaoyao 31, 241–243.