Cytotoxic phenolic compounds in leaf buds of *Populus tremuloides*

André Pichette, Azadeh Eftekhari, Patricia Georges, Serge Lavoie, Vakhtang Mshvildadze, and Jean Legault

Abstract: Phytochemical investigations of the EtOH extract of *Populus tremuloides* leaf buds led to the isolation of 19 phenolic compounds. Among them, (1*S*,2*S*)-1-[4-*O*-*E*-coumaroyl-β-D-glucopyranosyloxy]cyclohexanediol was reported for the first time, and its structure was determined by spectroscopic (NMR and MS) and chemical methods. Seventeen of the isolated compounds were tested for their cytotoxicity against lung carcinoma (A549) and colorectal adenocarcinoma (DLD-1) human cell lines. Antibacterial activity was also evaluated against *Escherichia coli* and *Staphylococcus aureus*.

Key words: quaking aspen, coumarate, flavonoids, NMR.

Résumé : Une étude phytochimique d'un extrait éthanolique des bourgeons de feuilles du *Populus tremuloides* a permis d'isoler 19 composés phénoliques, dont le (1*S*,2*S*)-1-[4-*O*-*E*-coumaroyl-β-D-glucopyranosyloxy]cyclohexanediol, un produit qui n'a jamais été rapporté antérieurement et dont la structure a été déterminée par des méthodes chimiques et spectroscopiques (RMN et spectrométrie de masse). Les propriétés cytotoxiques de 17 de ces produits on été évalué contre des lignées de cellules humaines de carcinome du poumon (A549) et d'adénocarcinome (DLD-1). Leur activité antibactérienne a aussi été évalué contre l'*Escherichia coli* et le *Staphylococcus aureus*.

Mots-clés : peuplier faux-tremble, coumarate, flavonoïdes, RMN.

Introduction

The buds exudates of many plant species of *Populus* genus are known as raw material processed by bees into propolis.[1] The latter product has been widely used in popular medicine as antibacterial,[2–4] anti-inflammatory,[5] antioxidant,[6,7] and cytostatic treatments.[8] The biological activity of propolis samples is mainly due to phenolic compounds like flavonoids, aromatic acids, and diterpenic acids,[9,10] which are the principal constituents of the buds of *Populus* species.[11–13] Recently, studies in the northern-type propolis showed a potential source of biologically active substances in *Populus tremuloides*,[14] which are widely spread across North America.[15] In spite of its use as ointment by Amerindian traditional medicine to treat numerous diseases, such as coughs, colds, and irritated nostrils,[16] few studies were carried out on the medicinal applications of *P. tremuloides*. In the present study, the isolation and structure elucidation of a new phenolic compound from *P. tremuloides* Michaux, along with 18 known products are described.

Results and discussion

The leaf buds of *Populus tremuloides* were extracted with EtOH and EtOH/H_2O under reflux. After evaporation of EtOH in vacuo, the aqueous phase was successively partitioned with hexane and *n*-BuOH. The *n*-BuOH soluble extract was purified on an open Diaion® column with a gradient of decreasing polarity, and three fractions were obtained. Each fraction was investigated for in vitro cytotoxic and antibacterial biological activities. Cytotoxic activity evaluations were carried out on human lung cancer (A549), human colorectal cancer (DLD-1), and normal skin fibroblasts (WS1) using the resazurin reduction test as previously described in the literature.[17] Antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The results (Table 1) show that the last fraction C was found to exert a weak cytotoxic activity against A549 (IC_{50}, 96 ± 7 μg mL^{-1}) and DLD-1 (IC_{50}, 89 ± 6 μg mL^{-1}), but was inactive toward bacterial cell lines. Thus, bioassay-guided fractionation of fraction C was undertaken with a combination of different chromatographic techniques leading to the isolation of a new compound **1** together with 18 known compounds: chaenomeloidin (**2**),[18] prunin (**3**),[19] echinaticin (**4**),[20] echinacin (**5**),[21] tremulacin (**6**),[22] salicine (**7**),[23] tremuloidin (**8**),[24] genkwanin (**9**),[25] rhamnocitrin (**10**),[26] sakuranetin (**11**),[27] acacetin (**12**),[28] kaempferide (**13**),[29] aromadendrin (**14**),[30] phenylmethyl coumarate (**15**),[31] phenethyl *p*-coumarate (**16**),[32] cinnamyl coumarate (**17**),[33] phenylmethyl caffeate (**18**),[34] and *trans*-ferulic acid (**19**)[35] (Fig. 1). Known compounds were identified by comparison of their spectroscopic data with the values found in the literature. NMR spectroscopic data for phenethyl *p*-coumarate (**16**), which was also isolated from buds of *P. tremuloides*, were not available. Therefore, complete ^1H and ^{13}C NMR spectroscopic data for **16** are also reported.

The molecular formula ($C_{21}H_{28}O_9$) of **1**, a white amorphous powder, was determined from its HR-ESI-MS spec-

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Table 1. In vitro cytotoxicity and antibiotic results of the Diaion® column's fractions.

	IC_{50} (μg mL^{-1})[a]			MIC[b]	
Samples	A549	DLD-1	WS1	*S. aureus*	*E. coli*
Fraction A	>100	>100	>100	>100	>100
Fraction B	>100	>100	>100	>100	>100
Fraction C	96±7	89±6	80±10	>100	>100
Etoposide[c]	2.8±0.5	2±1	>50	NT[d]	NT[d]
Chloramphenicol[c]	NT[d]	NT[d]	NT[d]	>5	0.37±0.06

Note: Mean values (± standard deviation) for triplicate assays.

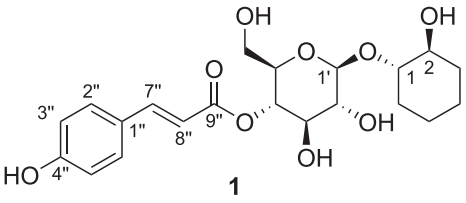
[a]Concentration of extract that caused 50% inhibition of cell proliferation.

[b]Minimum concentration of extract that resulted in inhibition of visible growth.

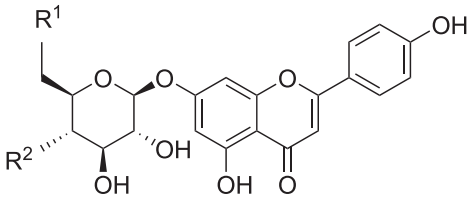
[c]Positive control.

[d]Not tested.

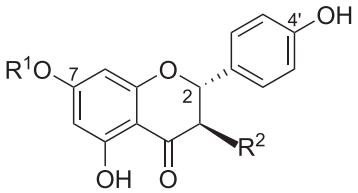
Fig. 1. Polyphenols from *Populus tremuloides*.



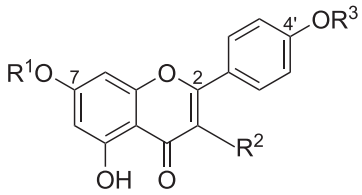
1



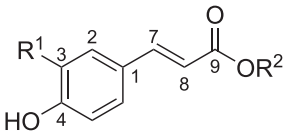
4 R^1 = OH; R^2 = Coumaroyl
5 R^1 = Coumaroyl; R^2 = OH



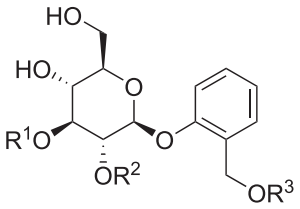
3 R^1 = Glc; R^2 = H
11 R^1 = CH_3; R^2 = H
14 R^1 = H; R^2 = OH



9 R^1 = CH_3; R^2 = H; R^3 = H
10 R^1 = CH_3; R^2 = OH; R^3 = H
12 R^1 = H; R^2 = H; R^3 = CH_3
13 R^1 = H; R^2 = OH; R^3 = CH_3



15 R^1 = H; R^2 = CH_2-Ph
16 R^1 = H; R^2 = CH_2-CH_2-Ph
17 R^1 = H; R^2 = CH_2-CH=CH-Ph
18 R^1 = OH; R^2 = CH_2-Ph
19 R^1 = OCH_3; R^2 = H



2 R^1 = CO-Ph; R^2 = H; R^3 = H
6 R^1 = H; R^2 = CO-Ph; R^3 =
7 R^1 = H; R^2 = H; R^3 = H
8 R^1 = H; R^2 = CO-Ph; R^3 = H

trum (positive-ion mode) on the basis of a quasimolecular ion peak at *m*/*z* 447.1623 $[M + Na]^+$ (calcd. 447.1631). Infrared absorption bands at 3328, 1602, 1160, 982, and 833 cm^{-1} suggested the presence of hydroxyl groups, aromatic system, and an ester carbonyl groups. ^{13}C NMR spectrum displayed 19 carbon signals (Table 2) separated by DEPT spectrum into five methylenes, seven aliphatic oxymethines, four unsaturated methines, and three quaternary carbons (one for an ester carbonyl). Among them, six resonances could be assigned to a sugar moiety. ^1H NMR spectrum confirmed the presence of a hexose moiety with anomeric proton at δ_H 4.45 (1H, d, J = 7.8 Hz). ^1H NMR spectrum also shows the presence of two *trans*-olefinic protons at δ_H 7.66 (1H, d, J = 15.9 Hz) and δ_H 6.37 (1H, d, J = 15.9 Hz) and a 1,4-disubstituted aromatic ring with four protons at δ_H 7.47 (2H, d, J = 8.6 Hz) and 6.81 (2H, d, J = 8.6 Hz). HMBC correlations at δ_H 7.47 (H-2″, H-6″) and δ_C 147.3 (H-7″), δ_H 6.81 (H-3″, H-5″) and δ_C 161.5 (C-4″), and δ_H 7.66 (H-7″) and δ_C 168.6 (C-9″) suggested the presence of a coumaroyl moiety. Analysis of the COSY, HSQC, and HMBC spectra led to the identification of a third aglycone sytem: the cyclohexane-1,2-diol. HMBC correlation between the methine proton at δ_H 4.87 (H-4′) and the carbonyl group at δ_C 168.6 (C-9″) suggested the linkage between the glucose and the coumaroyl moiety. Finally, the correlation between δ_H 4.45 (H-1′) and δ_C 79.4 (C-1) indicated the linkage site of the glucose moiety to the aglycone.

Acidic hydrolysis of **1** and TLC analysis of the aqueous phase afforded identification of glucose as the sugar component. Absolute configuration of the glucose as D was determined by optical rotations in comparison with authentic standard. The presence of *p*-coumaric acid in the organic phase was confirmed by TLC in comparison with authentic standard. Cyclohexane-1,2-diol was also detected in the organic phase using GC–MS and NMR analysis.[36] The absolute configuration of cyclohexane-1,2-diol could be determined directly from the organic phase, since the other aglycon part, namely, *p*-coumaric acid, is optically inactive. The organic phase showed positive value in optical activity measurement meaning that (1*S*,2*S*)-cyclohexane-1,2-diol has been isolated.[37] The structure of **1** was thus confirmed as (1*S*,2*S*)-1-[4-*O*-*E*-coumaroyl-β-D-glucopyranosyloxy]cyclohexanediol.

Compound **16** has been identified by many authors, but surprisingly, no complete NMR assignation was given.[38] Therefore, complete ^1H and ^{13}C characterization was accomplished using ^1H, ^{13}C, and 2D spectra (Table 3). First, the same *p*-coumaroyl moieties as in **1** were identified with δ_H at 6.29 (1H, d, J = 15.9 Hz, H-8), 6.85 (2H, d, J = 8.1 Hz, H-3 and H-5), 7.42 (2H, d, J = 8.1 Hz, H-2 and H-6), and 7.62 (1H, d, J = 15.9 Hz, H-7) and δ_C at 115.5 (C-8), 115.9 (C-3 and C-5), 127.3 (C-1), 130.0 (C-2 and C-6), 144.6 (C-7), 157.7 (C-4), and 167.4 (C-9). Additionally, five overlapped ^1H NMR signals between δ_H 7.20–7.30 along with two methylene triplets at 3.02 (2H, t, J = 6.9 Hz, H-7′) and 4.42 (2H, t, J = 6.9 Hz, H-8′) were attributed to a phenethyl moiety. The HMBC correlation between H-8′ and C-9 confirmed the link between the phenethyl and the *p*-coumaroyl groups.

Compounds **10** and **13** were isolated as a mixture. Separation of each constituent was not performed due to their small amounts. Therefore, careful examination of NMR spectra (^1H, ^{13}C, DEPT, and HSQC) and comparison with literature allowed the identification of these components as rhamnocitrin (**10**)[29] and kaempferide (**13**).[26] Moreover, the biological results were obtained using commercial pure products. Because of the low isolated yields of compounds **3** and **19**, those compounds were also tested from commercial materials.

All isolated compounds, except compounds **2** and **4**, were evaluated using resazurin reduction test for their cytotoxicity against human lung cancer (A549), human colorectal cancer

Table 2. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data for compound **1** in methanol-d_4.

Position	δ_C (multiplicity)[a]	δ_H (multiplicity, J in Hz)
1	79.4 (*d*)	3.87 (*m*)
2	71.0 (*d*)	3.85 (*m*)
3	31.5 (*t*)	1.79 (*m*)
		1.55 (*m*)
4	22.4 (*t*)	1.65 (*m*)
		1.34 (*m*)
5	23.1 (*t*)	1.71 (*m*)
		1.31 (*m*)
6	27.6 (*t*)	1.82 (*m*)
		1.63 (*m*)
1′	102.2 (*d*)	4.45, (*d*, 7.8)
2′	75.1 (*d*)	3.35 (*dd, 9.3, 7.8*)
3′	75.6 (*d*)	3.65 (*t, 9.3*)
4′	72.5 (*d*)	4.87 (*m*)
5′	76.1 (*d*)	3.53 (*m*)
6′	62.4 (*t*)	3.62 (*dd, 15.0, 5.4*)
		3.54 (*m*)
1″	127.2 (*s*)	
2″, 6″	131.3 (*d*)	7.47 (*d*, 8.6)
3″, 5″	116.9 (*d*)	6.81 (*d*, 8.6)
4″	161.5 (*s*)	
7″	147.3 (*d*)	7.66 (*d*, 15.9)
8″	114.8 (*d*)	6.37 (*d*, 15.9)
9″	168.6 (*s*)	

[a]Multiplicities were deduced from DEPT experiments.

Table 3. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data for compound **16** in methanol-d_4.

Position	δ_C (multiplicity)[a]	δ_H (multiplicity, J in Hz)
1	127.3 (*s*)	
2, 6	130.0 (*d*)	7.42 (*d*, 8.1)
3, 5	115.9 (*d*)	6.85 (*d*, 8.1)
4	157.7 (*s*)	
7	144.6 (*d*)	7.62 (*d*, 15.9)
8	115.5 (*d*)	6.29 (*d*, 15.9)
9	167.4 (*s*)	
1′	137.9 (*s*)	
2′, 6′	128.9 (*d*)	7.26 (*m*)
3′, 5′	128.5 (*d*)	7.32 (*m*)
4′	126.6 (*d*)	7.25 (*m*)
7′	35.2 (*t*)	3.02 (*t*, 6.9)
8′	65.0 (*t*)	4.42 (*t*, 6.9)

[a]Multiplicities were deduced from DEPT experiments.

(DLD-1), and normal skin fibroblasts (WS1).[17] Results presented in Table 4 are expressed as the concentration of product inhibiting cell growth by 50% (IC_{50}). Etoposide was used as positive control with IC_{50} of 2.8 and 2.0 μmol/L against A549 and DLD-1 cell lines, respectively. The phenolic compounds were regarded as active when the IC_{50} was smaller than 100 μmol/L.[39] The compound **9** was found to be the most active with IC_{50} ranging from 5.8 to 9.2 μmol/L. Moreover, compounds **10**, **12**, and **15** were moderately active against cancer cells with IC_{50} ranging from 19 to 37 μmol/L. In contrast to compounds **9**, **12**, and **15**, compound **10** was significantly selective toward cancer cells with IC_{50} of 31 μmol/L for A549 and 37 μmol/L for DLD-1 in comparison with 87 μmol/L for normal cells, WS1. Although the cytotoxicity of compounds **9** and **12** were known on A549 cells,[40,41] the activity on human colorectal adenocarcinoma DLD-1 was never reported. Finally, compounds **13** and **18** were found weakly cytotoxic, and all the other compounds tested were inactive. As far as structure–activity relationships are concerned, these in vitro results suggest that the addition of a double bond in C-2 position in molecule **9,** with regard to compound **11,** increases the cytotoxic activity. Similarly, the presence of a methoxy group in C-7 position and a hydroxyl in C-4′ position in the flavone **9** seem to have a beneficial effect on the cytotoxic activity in comparison with the acacetin (**12**), where the inversion of these groups reduces the activity. On the other hand, the presence of hydroxyl group in R^2 of compounds **10** and **13** is detrimental for the activity in comparison with compounds **9** and **12**, respectively. In the case of compounds **15**–**19**, only molecules bearing a benzyl group exhibited cytotoxicities (**15** and **18**). Moreover, the hydroxyl group in R^1 of compound **18** reduces significantly the cytotoxicity in comparison with **15**. All compounds were also evaluated for their antibacterial activities against *S. aureus* and *E. coli*, but no significant activity was observed.

Concluding remarks

In conclusion, the structure of a new compound **1** was described and 19 compounds were identified from *P. tremuloides*. Among them, compounds **2** and **5** were reported for the first time in *Populus* genus and compounds **3**, **4**, **9**, **10**, **12**, **16**, and **17** for the first time in *P. tremuloides*. Compound **9** was found to be the most cytotoxic against lung carcinoma cell (A549) and colorectal adenocarcinoma (DLD-1) human cell lines. Interestingly, compound **10** was selective toward both cancer cell lines in comparison with normal cells. Finally, all compounds tested do not possess antibacterial activity.

Experimental

General

Optical rotations were measured with an automatic polarimeter Rudolph Research Analytical Autopol IV. FTIR spectra were recorded with a PerkinElmer SpectrumOne. High resolution electrospray ionization mass spectrum was conducted in positive mode with an Applied Biosystems/MDS Sciex QSTARXL QqTOF MS system. 1D and 2D NMR spectra (^1H–^1H COSY, HSQC, and HMBC) were performed using an Avance 400 Bruker spectrometer equipped with a 5 mm QNP-probe. Chemical shifts were expressed in δ (ppm) units relative to TMS as an internal standard, and coupling constants were given in Hz. Preparative HPLC was performed on an Agilent 1100 liquid chromatography system, equipped with a solvent delivery system, an autosampler and a UV-MWD detector. Samples were eluted in an Intertsil prep-ODS column C18 (20 × 250 mm; 10 μm) at room temperature with a flow rate of 10 mL min^{-1}. GC–MS analyses were performed with an instrument (Agilent Technologies 6890N) fitted with a mass-selective detector (Agilent Technologies 5973), a split–splitless injection port, and an apolar capillary column DB-5MS (30 m × 0.25 mm × 0.25 μm).

Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254}, 0.25 mm pre-coated TLC plates (Silicycle, Québec, Canada). Flash column chromatographies (CC) were performed on silica gel (40–63 μm with indicator F_{254}, Silicycle, Québec, Canada) and on C_{18} reversed-phase silica gel (carbon 11%, 40–69 μm, Silicycle, Québec, Canada). Polyamide CC-6 was purchased from Macherey-Nagel (Germany) and Diaion HP-20 from Supelco. Detection of the phenolic compounds was carried out by spraying TLC plates with polyethylene glycol (NP/PEG) reagent followed by heating at 110 °C and detected by UV absorption at 254 and 365 nm. TLC identification of monosaccharides was performed with CH_2Cl_2/MeOH/H_2O (50:25:5) solvent system. The compounds were visualized by spraying an orthophosphoric acid solution of naphtoresorcinol 5% in EtOH, followed by heating at 110 °C.

The commercial samples used for biological tests, namely, prunin (**3**), kaempferide (**13**), and *trans*-ferulic acid (**19**), were purchased from Indofine Chemical Company (USA). Rhamnocitrin (**10**) was purchased from Apin Chemicals Ltd. (UK).

Plant material

Leaf buds of *P. tremuloides* Michaux were collected in the boreal forest to the south of Chicoutimi, Québec, Canada, in April 2006. Samples were identified by Patrick Nadeau (Département des sciences fondamentales, Université du Québec à Chicoutimi). A voucher specimen (QFA-A540466) was deposited at the Herbarium Louis-Marie of Université Laval, Québec, Canada.

Extraction and isolation

The buds of *P. tremuloides* (1 kg) were exhaustively extracted with EtOH (3 L, 60 °C, three times, 2 h each time) followed by EtOH/H_2O (7:2). The extracts were filtered and pooled. After evaporation of EtOH in vacuo, the aqueous phase was extracted successively with hexane (500 mL × 5) and saturated *n*-BuOH with H_2O (500 mL × 5). The *n*-BuOH phase was decanted and evaporated in vacuo. The residue (80 g) was fractionated using an open Diaion® column eluted with H_2O/MeOH with 30%, 50%, and 80% of MeOH. Three fractions were obtained: A (6.46 g), B (7.24 g), and C (58.96 g).

Fraction C was purified on silica gel CC, eluted with $CHCl_3$/MeOH gradient (60:1 → 5:1, *v*/*v*), and three fractions were obtained: C1, C2, and C3.

Fraction C1 (16.73 g) was subjected to silica gel using a gradient of $CHCl_3$/MeOH (90:1→60:1, *v*/*v*) as eluent. Subfraction C1.1 (384 mg), obtained from $CHCl_3$/MeOH

Table 4. In vitro cytotoxicity results of isolated compounds (**1**–**19**).

	IC_{50} (μmol/L ± SD)[a]		
Compounds	A549	DLD-1	WS1
1	>100	>100	>100
2	NT[b]	NT[b]	NT[b]
3	>100	>100	>100
4	NT[b]	NT[b]	NT[b]
5	>100	>100	42±4
6	>100	>100	>100
7	>100	>100	>100
8	81±3	>100	>100
9	9±3	9.2±0.9	5.8±0.3
10	31±2	37±3	87±3
11	>100	>100	>100
12	27±3	23±6	20±2
13	60±10	>100	42±5
14	>100	>100	>100
15	19±2	19.2±0.9	26±3
16	>100	>100	>100
17	>100	>100	>100
18	45.8±0.9	39±3	51±7
19	>100	>100	>100
Etoposide[c]	2.8±0.5	2±1	>50

Note: Mean values (± standard deviation) for triplicate assays.

[a]Concentration that caused 50% inhibition of cell proliferation.

[b]Not tested.

[c]Positive control.

(90:1), was separated on silica gel CC with $CHCl_3$/MeOH (80:1) as eluent, to give three fractions: C1.1A, C1.1B, and C1.1C. Subfraction C1.1A was purified by preparative HPLC with a gradient elution of MeOH/H_2O (50:50 → 85:15, *v*/*v*) yielding compounds **15** (249 mg), **16** (5 mg), and **17** (41 mg). Subfraction C1.1B (2.36 g) was applied successively on a silica gel CC and a reversed-phase CC using MeOH/H_2O gradient (50:50 → 70:30, *v*/*v*) as eluent to give **11** (160 mg). Compound **9** (3 mg), **12** (18 mg), and a mixture of **10** and **13** (27 mg) were isolated after a silica gel CC eluted with $CHCl_3$/MeOH (90:1) and a preparative HPLC (isocratic CH_3CN/H_2O 40:60) of fraction C1.2. Compound **18** (50 mg) was obtained from fraction C1.3 (294 mg) after repeated silica gel CC ($CHCl_3$/MeOH, 75:1) and polyamide flash column (MeOH/H_2O, 50:50 → 75:25).

Fraction C2 was chromatographed on silica gel CC with a gradient elution of $CHCl_3$/MeOH (75:1 → 15:1, *v*/*v*) to give eight fractions: C2.1–C2.8. Subfraction C2.7 (558 mg) was separated by preparative HPLC using an isocratic mobile phase of CH_3CN/H_2O/HCOOH (40:60:1) to afford **2** (19 mg) and **14** (3 mg).

Fraction C3 (5.4 g) was purified on silica gel using a gradient of $CHCl_3$/MeOH (25:1 → 7:1) for elution to give five subfractions: C3.1–C3.5. C3.2 was separated on silica gel CC with $CHCl_3$/MeOH (20:1) giving **6** (289 mg). Some purifications on different silica gel CC of subfraction C3.2.1 permitted to obtain **19** (2 mg). Subfraction C3.4 (418 mg) was separated by preparative HPLC using an isocratic mobile phase of CH_3CN/H_2O (30:70) to afford compounds **1** (20 mg), **3** (8 mg), **4** (14 mg), and **8** (37 mg). Subfraction C3.5 (482 mg) was separated by HPLC using a gradient of MeOH/H_2O (10:90 → 100:0) to give **5** (21 mg) and **7** (35 mg).

(1*S*,2*S*)-1-[4-*O*-*E*-coumaroyl-β-D-glucopyranosyloxy]cyclohexanediol (1)

White amorphous powder. $[\alpha]_D^{25}$ –35.3° (*c* 1.0, MeOH). IR (neat) ν_{max}: 3328, 2935, 1696, 1602, 1160, 1080, 1024, 982, and 833 cm^{-1}. ^1H and ^{13}C NMR spectroscopic data: see Table 2. HR-ESI-MS *m*/*z*: 447.16225 $[M + Na]^+$ (calcd. for $C_{21}H_{28}O_9Na$: 447.16310).

Acid hydrolysis of 1

Compound **1** was dissolved in HCl 10% and heated at 110 °C for 4 h. The resulting hydrolysate was extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The presence of *p*-coumaric acid was confirmed with standard sample on TLC ($CHCl_3$/MeOH 10:1 as eluent and developing with NP/PEG reagent). The presence of cyclohexane-1,2-diol in the organic phase ($[\alpha]_D$ + 3.4) was confirmed with a GC–MS analysis: Injector temperature, 250 °C; ionization voltage, 70 eV (EI-MS); column temperature, 40 °C for the initial 2 min followed by an increase of 15 °C min^{-1} up to 350 °C; carrier gas, He; column flow rate, 1 mL min^{-1}. Cyclohexane-1,2-diol was detected at R_t 7.55 min. The aqueous phase was neutralized with *N*,*N*-dioctylmethylamine (10% in $CHCl_3$), and the solvents were evaporated under reduced pressure. The residue contained the monosaccharide D-glucose ($[\alpha]_D$ + 24.8).

Cell lines and culture conditions

Lung carcinoma (A549), colorectal adenocarcinoma (DLD-1), and normal skin fibroblast (WS1) human cell lines were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in minimum essential medium containing Earle's salts and L-glutamine (Mediatech Cellgro, VA) to which were added 10% fetal bovine serum (Hyclone), vitamins (1X), penicillin (100 IU mL^{-1}) and streptomycin (100 μg mL^{-1}), essential amino acids (1X), and sodium pyruvate (1X) (Mediatech Cellgro, VA). Cells were kept at 37 °C in a humidified environment containing 5% CO_2.

Cytotoxicity assay

Exponentially growing cells were plated in 96-well microplates (BD Falcon) at a density of 5×10^3 cells per well in 100 μL of culture medium (DMEM with 10% SVF) and were allowed to adhere for 24 h before treatment. Increasing concentrations of each compound in MeOH or DMSO were then added (100 μL per well), and the cells were incubated for 48 h. The final concentration of MeOH or DMSO in the culture medium was maintained at 0.25% (*v*/*v*) to avoid solvent toxicity. Cytotoxicity was assessed using resazurin[17] on an automated 96-well Fluoroskan Ascent F1™ plate reader (Labsystems) using excitation and emission wavelengths of 530 and 590 nm, respectively. Fluorescence was proportional to the cellular metabolic activity in each well. Survival percentage was defined as the fluorescence in experimental wells compared to that in control wells after subtraction of blank values. Each experiment was carried

out three times in triplicate. IC_{50} results were expressed as means ± standard deviation.

Antibacterial assays

Antibacterial activity was evaluated using the microdilution method[42] but with some modifications: exponentially growing bacteria were plated in 96-well flat bottom microplates (BD Flacon) at a density of 5×10^3 Gram-negative *E. coli* (ATCC 25922) or 40×10^3 Gram-positive *S. aureus* (ATCC 25923) per well in 100 μL nutrient broth (Difco). The concentration of ethanol in the culture medium was maintained at 0.25% (*v*/*v*) to avoid solvent toxicity. Fifty microliters of 4% resazurin was added to each well, and the microplates were incubated for 6 h at 37 °C. Fluorescence was measured after 6 h on an automated 96-well Fluoroskan Ascent Fl™ plate reader (Labsystems) using excitation and emission wavelengths of 530 nm and 590 nm, respectively.

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