Cytotoxic Amides from Fruits of Kawakawa, *Macropiper excelsum* *

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- amides
- *Macropiper excelsum*
- Piperaceae
- cytotoxicity
- synthesis

Abstract

Cytotoxic amides have been isolated from the fruits of the endemic New Zealand medicinal plant kawakawa, *Macropiper excelsum* (Piperaceae). The main amide was piperchabamide A and this is the first report of this rare compound outside the genus *Piper*. Eleven other amides were purified including two new compounds with the unusual 3,4-dihydro-1(2H)-pyridinyl group. The new compounds were fully characterized by 2D NMR spectroscopy, which showed a slow exchange between two rotamers about the amide bond, and they were chemically synthesized. In view of the antitumor activity of the related piperlongumine, all of these amides plus four synthetic analogs were tested for cytotoxicity. The most active was the piperine homolog piperdardine, with an IC_{50} of 14 µM against HT 29 colon cancer cells.

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Introduction

The islands of Aotearoa New Zealand were the last major land mass on Earth to be settled by humans. Māori settlers arrived from Polynesia to find a densely forested land, with almost no species that they would have encountered previously, as the flora of New Zealand is about 80% endemic [1]. However, the Māori recognized the relationship of one plant to the Polynesian kava, *Piper methysticum*, and named it kawakawa [2]. Kawakawa is classified as *Macropiper excelsum* (G.Forst.) Miq. (Piperaceae), a New Zealand endemic in a small *Piper*-related genus that also contains eight other species across the Pacific [3]. There are two recognized subspecies of *M. excelsum* [3], ssp. *psittacorum* (Endl.) A.C. Sm. found on Lord Howe Island, Norfolk Island, Kermadec Island, and smaller islands off the north coast of the North Island of New Zealand, and the main ssp. *excelsum*, which grows as a shrub or small tree on the North Island and warmer parts of the South Island of New Zealand [4].

Māori developed a wide range of medicinal and food uses for kawakawa, especially pain relief [2, 5]. Leaves were usually used for medicinal purposes, but the fruits were also eaten "rejecting the numerous seeds" [5]. Extracts of leaves showed antimicrobial activity [6]. The roots were also used medicinally [5], but they do not contain the narcotic kavalactones (Plant & Food Research unpublished results) found in kava roots [7–9].

The leaves of kawakawa contain myristicin as the main volatile component [10] plus other volatile phenyl propanoids, and mono- and sesquiterpenes [11]. A rare bioactive lignan, diayangambin, has been found in both wood [12] and leaves [13]. This compound exerts immunosuppressive and anti-inflammatory effects *in vivo* [14]. We could find no reports of kawakawa fruit chemistry, despite fruits of other Piperaceae being rich sources of bioactive amides such as pungent piperine **1** [15–17] and the antitumor piperlongumine/piplartine **2** (structures in **Fig. 1**) [18, 19].

The fruits of female kawakawa (*M. excelsum* is dioecious [20]) are compound spikes (drupes) carrying up to 150 seeds each [21]. They develop from being green and rigid to orange and soft during ripening [22]. We now report the identification of 12 amides from these fruits, including the rare piperchabamide **3** and two new variants, **4** and **5**. The new compounds and four related com-

* Dedicated to Professor Dr. Dr. h. c. mult. Adolf Nahrstedt on the occasion of his 75th birthday.

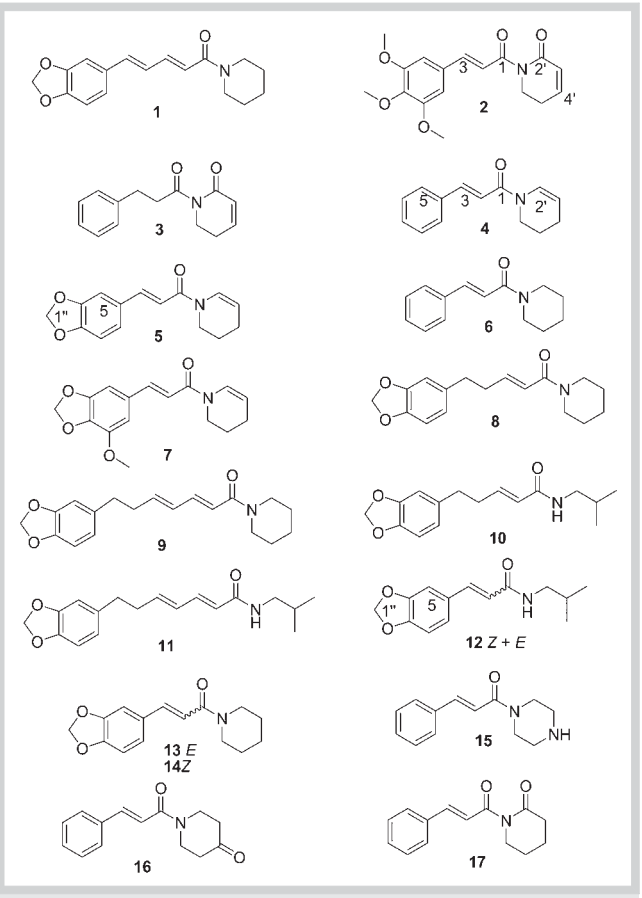

Fig. 1 Structures of kawakawa amides and related compounds.

pounds have been synthesized, and the cytotoxic activities of the natural and synthetic amides are reported.

Results and Discussion

Ethanol extracts of kawakawa leaves and fruits were analyzed by reversed-phase liquid chromatography (RPLC). The leaf extract showed myristicin as the major component with diayangambin also present, as expected from previous reports [10, 13]. The fruit extract showed a contrasting composition, with diayangambin as the major component, a trace of myristicin, plus a series of other UV active peaks. A bulk extract of kawakawa fruits was fractionated by RP and silica gel column chromatography, and then preparative RPLC to give the main unknown component. HR-ESI-MS supported a molecular formula of $C_{14}H_{15}NO_2$ (**Table 1**). 2D NMR spectra (data not shown) led us to structure **3**, which has been reported as piperchabamide A with chemical shift data matching ours [23]. Piperchabamide A **3** has only been reported from *Piper chaba* [23] and *Piper capense* [24], and this is the first report of any amides from *Macropiper*.

Two other amides (**4** and **5**) purified from this kawakawa fruits extract showed unusual features in their NMR spectra and could not be matched to any known *Piper* amides [17, 25]. The ^{1}H and ^{13}C NMR spectra of the main unknown **4** (**Table 2**) showed pairing of some signals, suggesting the presence of two closely related compounds in a ratio of about 3 : 1, even though the RPLC analyses showed only one sharp peak. However, in the phase-sensitive 2D NOESY spectrum, two sorts of cross-peaks were present: in phase cross-peaks between the paired signals and opposite phase cross peaks corresponding to the usual through space NOE correlations. This effect was assigned to the separation of chemical exchange and cross-relaxation effects described by Davis and Bax [26], and the 2D NMR structure solution was completed on the two sets of signals.

HR-ESI-MS (**Table 1**) supported a molecular formula of $C_{14}H_{15}NO$ for **4**. The NMR data (**Table 2**) and COSY and HMBC spectra were appropriate for a cinnamoyl moiety, which was confirmed by closely matching data (**Table 2**) to those of cinnamoyl piperidine **6** [27]. The remaining amide portion showed connectivity and shifts of a 3,4-dihydro-1(2H)-pyridinyl group, which was supported by our data (**Table 2**) closely matching the two sets of signals reported for similar compounds [28]. This gave the proposed structure **4**, with two slowly exchanging rotamers (**Fig. 2**). Increasing the temperature gave a broadening of the ^{1}H NMR signals, most obviously H3′ (**Table 2**), but coalescence was not reached at the temperatures possible in $CDCl_3$ or $(CD_3)_2CO$.

The 3,4-dihydro-1(2H)-pyridinyl (piperideide) group is rare in natural amides, with only one previous example (**7**) from Piperaceae plants [29]. Surprisingly, only one set of NMR signals was listed for **7**, closely matching our data for the major rotamer of **4** (**Table 2**, the NMR spectra of **7** were in the same $CDCl_3$ solvent, but field strength and temperature were not stated) [29]. Patil et al. also reported a crystal structure for **7**, with the *E* conformation about the amide bond (**Fig. 2**). However, one cannot assume "… that the molecular shape observed in a crystal is identical to that to be found in fluid media." [30]; see for example [31].

Other reports of piperideide natural products have been from plants in the family Asteraceae, e.g., [28, 32]. Hofer analyzed the solution conformations of piperideides and found that the *E* conformation about the amide bond was slightly (60 : 40) more stable than the *Z* form (**Fig. 2**) [28]. Our NOESY results were not clear-cut because of small chemical shift differences between key signals, but did support the *E* form of **4** as the major conformation (**Fig. 2**).

The minor amide **5** showed a molecular formula of $C_{15}H_{15}NO_3$ by HR-ESI-MS (**Table 1**), i.e., with an additional CO_2 compared with **4**. The ^{1}H NMR spectrum of **5** showed a dioxymethylene substituted aromatic ring (**Table 2**), which accounted for these additional atoms, but was otherwise very similar to that of **4**, including the pairing of corresponding signals. However, this compound **5** was somewhat unstable, so it was synthesized for full characterization and for biological testing (see below).

During the re-isolation of compounds **4** and **5**, nine known amides were purified from kawakawa fruits, and identified by HR-ESI-MS and by matching ^{1}H NMR data with published spectra (**Table 1**). These compounds (**Fig. 1**) contain other combinations of amine and acid groups (**Fig. 1**): piperidine with piperidic acid (piperine **1**) or reduced (**8**) or homologous (**9, 13, 14**) acids and isobutylamine with some of the same acids (**10, 11, 12**). Such combinatorial diversity of natural amides is common within *Piper* species [25].

Because of the instability of the new amides **4** and **5**, and their low yield from kawakawa fruits, we decided to synthesize them. Commercially available cinnamoyl chloride readily reacted with three off-the-shelf piperidines to give **6, 15**, and **16** (all known, see **Table 1** and **Table S1**, Supporting Information) for structure-activity relationships (see below). 1,2,3,4-Tetrahydropyridine is not commercially available, so this approach to the syn-

Table 1 Amides isolated from fruits of kawakawa, *M. excelsum*, plus synthetic analogs.

Compound	Registry number	Source[a]	HR-ESI-MS (Da)[b]	NMR match	RPLC (min)[c]	Assay purity (%)[d]
Piperchabamide A **3**	807618-20-8	N	252.0992 ($C_{14}H_{15}NNaO_2$ req 252.0995)	[23]	11.2[f]	> 99
Piperideide **4**	New	N, S	236.1033 ($C_{14}H_{15}NNaO$ req 236.1046)	–	11.2[f]	> 99
Piperideide **5**	New	N, S	280.0923 ($C_{15}H_{15}NNaO_3$ req 280.0944)	–	11.2[f]	> 99
Piperine **1**	94–62–2	N, C	308.1231 ($C_{17}H_{19}NNaO_3$ req 308.1263)	[42]	11.3	> 99
Piperanine **8**	23512–46–1	N	310.1408 ($C_{17}H_{21}NNaO_3$ req 310.1414)	[42]	11.1	95
Piperdardine **9**	188426-70-2	N	336.1527 ($C_{19}H_{23}NNaO_3$ req 336.1570)	[43]	12.2	90
10	23512–53–0	N	298.1403 ($C_{16}H_{21}NNaO_3$ req 298.1414)	[44]	10.8	95
11	139906-29-9	N	324.1531 ($C_{18}H_{23}NNaO_3$ req 324.1570)	[45]	11.8	99
Fagaramide **12** 2 : 1 *Z* : *E*	*Z* 92449–51–9 *E* 495–86–3	N	270.1078 ($C_{14}H_{17}NNaO_3$ req 270.1101)	[46]	10.1	> 99
13	82857–82–7	N	282.1077 ($C_{15}H_{17}NNaO_3$ req 282.1101)	[47]	10.4	99
14	111479-04-0	N	282.1079 ($C_{15}H_{17}NNaO_3$ req 282.1101)	[24]	10.0	96
6	5422–81–1	S	NR[e]	[27]	10.6	> 99
15	55486–27–6	S	NR[e]	NF[f]	10.7	> 99
16	17077–45–1	S	NR[e]	NF[f]	8.4	> 99
17	188106-57-2	S	NR[e]	NF[f]	10.9	> 99

[a] N = natural product; S = synthetic; C= commercial; [b] $[M + Na]^+$; [c] Analytical RPLC retention time; [d] By RPLC; [e] not recorded; [f] not found, see **Table S1**, Supporting Information; [f] These compounds gave coincident peaks under the analytical RPLC conditions, but were separated under preparative RPLC conditions (**5**: 5.6 min; **3**: 5.9 min; **4**: 7.4 min)

thesis of the target compounds **4** and **5** was not feasible. Therefore, access to **4** was attempted from the known imide **17** [33]. Numerous attempts at forming the corresponding vinyl triflate of **17** were unsuccessful with only trace amounts observed. Instead, access was provided by the treatment of cinnamoyl chloride with α-tripiperideine [34] in the presence of triethylamine, affording **4** in a 39% yield. Under analogous reaction conditions, treatment of the known 3,4-methylenedioxycinnamoyl chloride [35] with α-tripiperideine provided **5** in a 47% yield. Both synthetic samples were spectroscopically identical to the natural products from kawakawa fruits.

The cytotoxicities of the full panel of kawakawa-derived compounds were tested in three cancer cell lines and in one "normal", non-transformed cell line. Various compounds showed some toxicity at 10 µM over 48 h, but the degree of toxicity varied between cell lines (**Figs. S5** and **S6**, Supporting Information). In the immortalized bone marrow-derived mesenchymal stem cell line RCB2157, quite a different effect was found. There was a complete lack of cytotoxicity with all compounds, although some did slightly increase cell growth rates. Therefore kawakawa-derived amides exhibit selectivity towards cancer cells. The highest toxicities were found using the HT29 colon cancer cell line, so IC_{50} values were determined for the three most active compounds.

Piperdardine **9** (IC_{50} 13.7 µM) and piperchabamide A **3** (IC_{50} 28.3 µM) both showed maximal toxicity at the highest concentration tested, but with piperine **1** (IC_{50} 70.3 µM), 38% of cells still retained viability at 400 µM. Piperdardine **9** has previously been reported to be cytotoxic to a range of other cell lines (as piperarboricoline, assayed at 64 µM) [36]. Piperchabamide A **3** has been evaluated for hepatoprotective [37] and antiparasitic [24] activites, but we could not find any previous reports of cytotoxic activity. In terms of the mechanism of action, piperlongumine **2** and analogs have been found to elevate cellular levels of reactive oxygen species selectively in cancer cell lines with the electrophilic functionalities O=C1–C2=C3 and O=C2′-C3′=C4′ (our numbering, **Fig. 1**), both important for activity [38]. Piperchabamide A **3** was the only amide tested with the O=C2′-C3′=C4′ functionality, whereas synthetic **17** is a regioisomer lacking O=C2′-C3′=C4′ but with O=C1–C2=C3 (**Fig. 1**). Compound **17** was less active than **3** against colon cancer cells, but more active than **3** against breast cancer cells. In prostate cancer cells, compound **17** caused a decrease in cell viability of 22.0 ± 4.9%, compared to the vehicle only-treated control, whereas **3** supported cancer cell growth, in-

	4		5	
Atom[b]	^{13}C	^{1}H	^{13}C	^{1}H
1A	164.3	–	164.4	–
1B	163.7	–	163.7	–
2A	117.0	6.91 (d, 15.5)	114.8	6.72 (d, 15.5)
2B	117.0	6.90 (d, 15.5)	114.8	6.70 (d. 15.5)
3A	143.2	7.68 (d, 15.5)	142.9	7.58 (d, 15.5)
3B	143.6	7.75 (d, 15.5)	143.4	7.64 (d, 15.5)
4A + 4B	135.2	–	129.5	–
5A + 5B	127.9	7.53 (br d, 8.0)	106.3	7.02 (d, 1.5)
6A + 6B	128.8	7.37 (m)	148.2[c]	–
7A + 7B	129.7	7.37 (m)	149.1[c]	–
8A + 8B	128.8	7.37 (m)	108.4	6.79 (d, 8.0)
9A	127.9	7.53 (br d, 8.0)	123.9	6.99 (br d, 8.0)
9B	–	–	124.0	6.99 (br d, 8.0)
2′A	125.3	6.83 (br d, 8.0)	125.3	6.81 (br d, 8.5)
2′B	124.7	7.30 (br d, 8.0)	124.6	7.28 (br d, 8.5)
3′A	108.8	5.02 (br dt, 8.0, 4.0)	108.6	4.99 (br dt, 8.0, 3.5)
3′B	109.5	5.16 (br m)	109.2	5.12 (br m)
4′	21.7	2.12 (m)	21.7	2.09 (m)
5′A	22.0	1.90 (m)	22.0	1.86 (m)
5′B	22.2		22.2	1.86 (m)
6′A	40.8	3.79 (m)	40.8	3.75 (m)
6′B	44.0	3.79 (m)	43.9	3.75 (m)
1′′	–	–	101.4	5.96 (s)

Table 2 NMR data of kawakawa amides **4** and **5**[a].

[a] In $CDCl_3$; ^{1}H at 500 MHz, shift in ppm (multiplicity, *J* in Hz); ^{13}C at 125 MHz, shift in ppm; [b] A = major rotamer, B = minor, see **Fig. 2**; [c] assignments uncertain

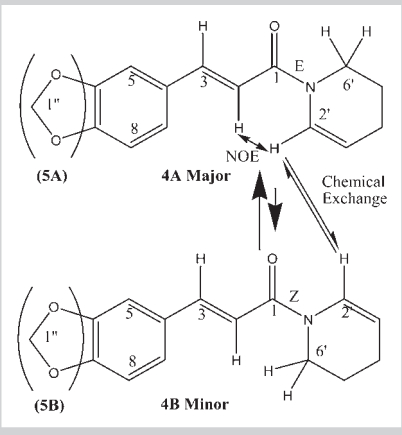

Fig. 2 Proposed rotamers of kawakawa amides **4** and **5**.

creasing viable cells by 9.3 ± 4.4%. (**Fig. S5**, Supporting Information).

In conclusion, we have shown the presence of bioactive amides in fruits of the endemic New Zealand medicinal plant kawakawa, *M. excelsum* (Piperaceae). The main amide was the rare piperchabamide A, which has selective cytotoxic activity.

Materials and Methods

General

IR spectra were recorded on a Bruker Optics Alpha FTIR spectrometer with ATR, and UV spectra on a Jasco spectrometer. High-resolution mass spectra (HR-MS) were recorded on a Bruker microTOFQ mass spectrometer using an electrospray ionization (ESI) source in the positive mode. ^{1}H, ^{13}C, and 2D NMR spectra were recorded at either 400 MHz on a Varian 400-MR NMR system or at 500 MHz on a Varian 500 MHz AR premium shielded spectrometer, from samples in $CDCl_3$ at 25 °C in 5 mm tubes. ^{1}H chemical shifts are reported relative to the residual $CHCl_3$ singlet at δ 7.25 ppm and ^{13}C chemical shifts relative to the $CDCl_3$ triplet at δ 77.00 ppm. CH_2Cl_2 and tetrahydrofuran (THF) were dried using a PureSolv MD-6 solvent purification system. EtOH, H_2O, and EtOAc were distilled before use and all other solvents and reagents were used as received. Analytical RPLC analyses (**Table 1**) were carried out using an Agilent HP1200, controlled with Agilent OpenLab software, at 20 °C on a C18 column [Phenomenex Luna ODS(3) 5 µm 100 Å 150 × 3 mm] with a 2 × 4 mm C18 guard column. Peaks were detected at 210, 254, and 280 nm. The mobile phase components were A – MeCN and B – H_2O, with 0.1% formic acid in both. A linear program was used: A $t_{0\,min}$ = 10%, $t_{12.5}$ = 100%, t_{15} = 100%, t_{16} = 10%, t_{20} = 10%. The flow rate was 0.5 mL/min, with an injection volume of 5 µL.

Plant material

Kawakawa fruits were collected from Parapara River Access Road, Golden Bay, Nelson, New Zealand (40°43′ 44″ E 172°40′ 21″ S) from January 23rd to 25th, 2008. Identification was by B.M.S., and a foliage voucher specimen (code 080125–07) is lodged in the Plant & Food Research Lincoln herbarium collection. Fruits were stored at − 80 °C until extracted.

Extraction and isolation

Frozen kawakawa fruits (43 g) were pulverized under liq N_2 and extracted with 96% EtOH (430 mL) overnight with shaking. The filtered extract was evaporated to dryness (35 °C) to give a green, sweet-smelling gum (2.43 g). This was bound onto C_{18} (octadecyl-functionalized silica gel, Aldrich, 2.5 g) and subjected to RP flash chromatography using a prepacked C_{18} column (Isolute, 10 g) preconditioned with EtOH, EtOH: H_2O (1 : 1), and H_2O. This was developed using H_2O, 1 : 4 EtOH : H_2O, 1 : 1 EtOH : H_2O, 4 : 1

EtOH : H_2O, EtOH, and EtOAc, two fractions per solvent mix. Fractions 7 and 8 eluted with 4 : 1 EtOH : H_2O were combined (433 mg) and subjected to silica gel flash chromatography (10 g Si gel 60, 200–400 mesh, 40–63 µm) on a column preconditioned with toluene (AR grade). The column was developed using a 1–10% gradient with toluene : EtOAc (lab grade distilled), collecting 26 fractions. Fractions 16–17 (18 mg) and 18–19 (11 mg) gave red spots at R_f 0.625 on Si TLC (Merck DC Kieselgel 60 F_{254}, mobile phase 7 : 3 toluene : EtOAc, visualized with vanillin H_2SO_4 dip) and were combined. The combined sample was subjected to preparative RPLC (Phenomenex Luna 5 µ C18 100, 250 mm × 10 mm, 5 µm; guard column Merck 100 RP 18 LichoCart 25 mm × 4 mm, 5 µm) with a 65 : 35 MeCN : H_2O mobile phase at 5 mL/min, 30 °C, 50 µL injections, and 210 and 254 nm detection. Three collected fractions gave piperideide **5** (2 mg, preparative RPLC RT 5.6 min), piperchabamide A **3** (6 mg, RT 5.9 min), and piperideide **4** (4 mg, RT 7.4 min)

Piperchabamide A **3**: colorless oil. ^1H and ^{13}C NMR data matching [23]; HR-ESI-MS, ◉ **Table 1**.

Piperideide **4**: colorless oil. UV (MeOH): λ_{max} 283 nm (ε 12 700); ^1H and ^{13}C NMR data, ◉ **Table 2**; HR-ESI-MS, ◉ **Table 1**.

Synthetic piperideide **4** *(see below)*: yellow crystals, m. p. 50 °C. UV (MeCN): λ_{max} 280 nm (ε 15 400); IR (film) 2956, 1641, 1602, 1416, 979, 761 cm^{-1}; ^1H and ^{13}C NMR data, ◉ **Table 2**; HR-ESI-MS, ◉ **Table 1**.

Piperideide **5**: colorless oil. ^1H NMR data, ◉ **Table 2**; HR-ESI-MS, ◉ **Table 1**.

Synthetic piperideide **5** *(see below)*: pale yellow crystals, m. p. 82 °C. UV (MeCN): λ_{max} 329, 293 nm (ε 18 600, 11 900); IR (film) 2902, 1637, 1595, 1488, 1446, 1250, 1031, 796 cm^{-1}; ^1H and ^{13}C NMR data, ◉ **Table 2**; HR-ESI-MS, ◉ **Table 1**.

Another batch of frozen kawakawa fruits (376 g) were pulverized under liq N_2 and extracted with $CHCl_3$ (1.75 L) overnight with shaking. The separated extract was evaporated to dryness to give a green gum (2.9 g). This was fractionated by various stages of silica gel flash chromatography and then preparative RPLC, as above, to give further samples of piperchabamide A **3** and piperideide **4**, plus the known amides piperine **1** and **8**–**14** identified in ◉ **Table 1**.

Syntheses of 4–6 and 15–17

Synthesis of piperideide **4**: Triethylamine (1.7 mL, 12.0 mmol) and dodecahydro-4a,8a,12a-triazatriphenylene (0.5 g, 2.0 mmol) were added to a solution of cinnamoyl chloride (1.0 g, 6.0 mmol) in THF (3 mL) [34]. The reaction mixture was stirred at 60 °C for 16 h before being diluted with H_2O (20 mL) and extracted with EtOAc (3 × 15 mL). The combined organic phase was washed with aqueous $NaHCO_3$ (sat.) (3 × 15 mL) and brine (15 mL), and then dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to provide the crude product as an orange oil (1.0 g). The crude product was purified using a silica gel column (20% EtOAc/40–60 pet ether) to give **4** (0.5 g, 39%, see properties above).

Synthesis of piperideide **5**: Triethylamine (0.5 mL, 3.2 mmol) and dodecahydro-4a,8a,12a-triazatriphenylene (150 mg, 3.2 mmol) were added to a solution of 3, 4-methylenedioxycinnamoyl chloride (335 mg, 1.6 mmol) in THF (1 mL). The reaction mixture was worked up and purified, as above, to give **5** (190 mg, 47%, see properties above).

Compounds **6, 15, 16**, and **17** were prepared by the reaction of cinnamoyl chloride with the appropriate amine to give **6** as a white solid: m. p. 121 °C (lit. 114–116 °C [39]), **15** as a white solid: m. p. > 260 = °C (lit. 80–81 °C [40]), **16** as a white solid: m. p. 91 °C (no lit. value found), and **17** as a white solid: m. p. 60 °C (lit. 58–59 °C [33]).

Cell lines and cell culture

MDA-MB-231 breast cancer and PC3 prostate cancer cells were a kind gift from Assoc. Prof. R. Rosengren, HT29 colon cancer cells were from CellBank Australia, and the immortalized bone marrow-derived mesenchymal stem cell line RCB2157 (MSC) was from the Riken BioResource Center through the National Bio-Resource Project of the MEXT, Japan. All cell lines were cultured in DMEM with 5% or 10% (for the MSCs) heat-inactivated FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were incubated at 37 °C in humidified conditions with 5% CO_2.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The quantity of cells remaining 48 h after treatment of the cells with the compounds or DMSO only (vehicle control) was compared using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which assesses mitochondrial and endoplasmic reticulum dehydrogenase activity [41]. MTT was made up to 5 mg/mL in PBS, added to cells at a final concentration of 0.5 mg/mL, and incubated at 37 °C for 3 h. DMSO was used to dissolve the formazan crystals and the resulting absorbance was read at 560 nm. The positive control chemotherapeutic drug cisplatin (Sigma-Aldrich, purity ≥ 99.9%) was tested at the 48 h time point for all cell lines. IC_{50} values were calculated as follows: HT29 cells: 17.8 µM; MDA-MB-231 cells: 3.7 µM; PC3 cells: 4.4 µM and MSC cells 3.8 µM.

Statistical analysis

The results are presented as mean ± standard deviation (SD) of three independent experiments. Absorbance values were normalized to vehicle only control (DMSO) for each experiment and a one-way ANOVA followed by a Dunnett's post hoc test was used to analyze the data. Statistical significance was determined as $p < 0.05$. To calculate IC_{50} values, concentrations were log transformed and the resulting graph analyzed by nonlinear regression.

Supporting information

^1H and ^{13}C NMR spectra of compounds **4** and **5**, cytotoxicity results against four cell lines, and ^1H NMR data for compounds **6, 15, 16**, and **17** are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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