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Activity of elaeochytrin A from *Ferula elaeochytris* on leukemia cell lines

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ABSTRACT

Phytochemical investigation of the roots of *Ferula elaeochytris* made it possible to isolate two sesquiterpene esters, 6-anthraniloyljaeschkeanadiol (elaeochytrin A) and 4β-hydroxy-6α-(*p*-hydroxybenzoyloxy)dauc-9-ene (elaeochytrin B), as well as eight known compounds: 6-angeloyljaeschkeanadiol, teferidin, ferutinin, 6-(*p*-hydroxybenzoyl)epoxyjaeschkeanadiol, 6-(*p*-hydroxybenzoyl)lancerotriol, 5-caffeoylquinic acid, 1,5-dicaffeoylquinic acid and sandrosaponin IX. The cytotoxic activities of all compounds were investigated on K562R (imatinib-resistant) human chronic myeloid leukaemia and DA1-3b/M2$^{BCR-ABL}$ (dasatinib-resistant) mouse leukemia cell line. Elaeochytrin A was the most active compound on both cell lines (IC_{50} = 12.4 and 7.8 μM, respectively). It was also tested on non-resistant human promyelocytic leukemia cells (HL60, IC_{50} = 13.1 μM) and was not toxic to normal peripheral blood mononuclear cells up to 100 μM.

1. Introduction

The genus *Ferula* (Apiaceae), known to contain both useful (*Ferula asafoetida* L., *Ferula gummosa* Boiss. and *Ferula hermonis* Boiss.) and toxic (*Ferula communis* L.) plants (Eigner and Scholz, 1999; Sadraei et al., 2001; Colman-Saizarbitoria et al., 2006; Rubiolo et al., 2006), is interesting as far as human health is concerned. Cytotoxic and cancer preventing properties have been increasingly investigated in plants of this genus (Barthomeuf et al., 2006, 2008; Iranshahi et al., 2008). Sesquiterpene lactones have in particular proven to be cytotoxic on several cancer cell lines and seem to be a promising class of natural compounds in the fight against cancer (Suzuki et al., 2007).

Ferula elaeochytris Korovin is a species found in Turkey and Syria. Previous phytochemical work led to the isolation of several compounds (Miski et al., 1983), one of which, ferutinin, was later shown to have antiproliferative and cytotoxic properties on estrogene-dependent (Lhuillier et al., 2005) or independent (Macho et al., 2004; Poli et al., 2005) tumor cell lines. We undertook a new phytochemical investigation of the roots of *F. elaeochytris* which led to the isolation of ten compounds, two of which were formerly unknown. Their structures were determined and their cytotoxic activity was assessed.

2. Results and discussion

From the *n*-BuOH-soluble fraction of the MeOH extract of *F. elaeochytris* (see Section 3) three known compounds were isolated. Two of them were phenolic compounds; their structures were established by NMR, MS and by comparison with published data. They were identified as 5-caffeoylquinic acid (chlorogenic acid) (Pauli et al., 1998) and 1,5-dicaffeoylquinic acid (Maruta et al., 1995), respectively. The third one was a saponin, the structure of which was determined by NMR, including specific TOCSY and NOESY experiments on its sugar moieties, and ESI-MS^2 on the compound and its prosaponin (after alkaline hydrolysis), and identified as sandrosaponin IX (Sánchez-Contreras et al., 2000).

Seven compounds were isolated from the CH_2Cl_2-soluble fraction of a methanolic root extract of *F. elaeochytris* (see Section 3). Five of them were identified as known compounds by NMR, MS and comparison of their data with that already published: 6-angeloyljaeschkeanadiol, teferidin, ferutinin (Miski et al., 1983), 6-(*p*-hydroxybenzoyl)epoxyjaeschkeanadiol and 6-(*p*-hydroxybenzoyl)lancerotriol (Fraga et al., 1985). Two compounds (**1**, **2**) were thought to be new natural products.

Compound **1** (Fig. 1) displayed absorbance for amino (3482 and 3372 cm^{-1}) and ester (1678 cm^{-1}) groups in the IR spectrum. The EI mass spectrum showed an $[M]^{+\cdot}$ at *m*/*z* 357 and the HR-ESI-MS showed an $[M+Na]^+$ at *m*/*z* 380.2191, suggesting a molecular formula of $C_{22}H_{31}NO_3$ (calculated value for $C_{22}H_{31}NO_3Na$: 380.2202).

As daucane sesquiterpenoids had previously been reported in this species, NMR spectra were compared with data available for

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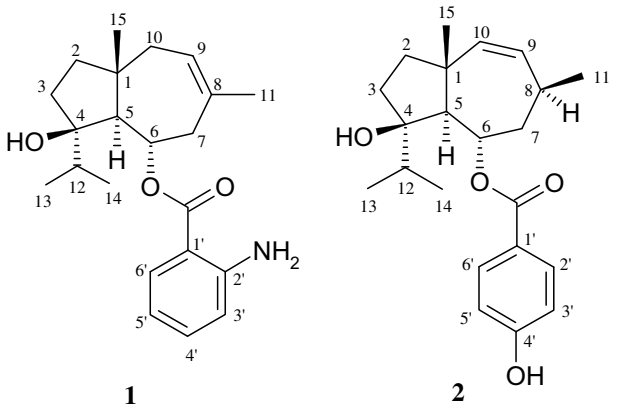

Fig. 1. Structures of new compounds isolated from *Ferula elaeochytris*.

this type of compound and most of the signals were close to those reported by Miski et al. (1983), suggesting that compound **1** had the same sesquiterpene moiety as they had found, that is to say jaeschkeanadiol. The ^{13}C NMR spectrum of **1** showed 22 signals, 15 of which were very similar to those reported by Chen et al. (2000) for the jaeschkeanadiol moiety of ferutinin. The seven remaining signals were attributed to an acyl moiety. In the ^{1}H NMR spectrum recorded in $CDCl_3$, characteristic methyl signals were observed at δ_H 0.87 (3H, *d*, *J* = 6.7 Hz) and 0.97 (3H, *d*, *J* = 6.7 Hz) which were correlated, in the COSY experiment, with a signal at δ_H 1.85, showing the presence of an isopropyl group. Two methyl signals corresponded to positions 11 (δ_H 1.83, *s*) and 15 (δ_H 1.12, *s*) of the molecule. Signals that were observed downfield were explained by a double bond between C-8 and C-9 (δ_H 5.56, 1H, *m*) and an oxygen atom next to H-6 (δ_H 5.30, 1H, *dt*, *J* = 10.2 and 3.1 Hz). The stereochemistry of the sesquiterpene moiety was determined as identical to that of jaeschkeanadiol by NOESY. Although the HMBC experiment showed a correlation of quaternary carbon (δ_C 168.4) with H-6, suggesting acylation at this position, the acyl group clearly differed from previous reports. Four signals were partly observed in the aromatic region of the ^{1}H NMR spectrum but two of them at δ_H 6.65 and 6.68 were overlapping. Another ^{1}H NMR spectrum was recorded in CD_3OD, which eliminated any overlapping of the signals: four signals were more clearly observed at δ_H 6.57 (*ddd*, *J* = 1.1, 7.0 and 8.2 Hz), 6.75 (*dd*, *J* = 1.0 and 8.3 Hz), 7.24 (*ddd*, *J* = 1.5, 7.0 and 8.4 Hz) and 7.74 (*dd*, *J* = 1.6 and 8.2 Hz), suggesting that the acyl group was an *ortho*-substituted benzoic acid. Direct comparison with published data (Krief et al., 2005) showed that it was *ortho*-aminobenzoic acid, i.e. anthranilic acid, which accounted for the uneven mass of compound **1** and the presence of an amino band in the IR spectrum; this was confirmed by alkaline hydrolysis followed by TLC analysis with an authentic sample of anthranilic acid. The structure of compound **1** was thus established as 6-anthraniloyljaeschkeanadiol (elaeochytrin A) and confirmed by bidimensional NMR experiments (COSY, HSQC, HMBC and NOESY).

In compound **2** (Fig. 1), acyl moiety was easily identified as *p*-hydroxybenzoic acid, by observing two doublets in the ^{1}H NMR spectrum at δ_H 7.89 (*J* = 8.6 Hz) and 6.85 (*J* = 8.6 Hz), which showed the *para* orientation of the hydroxyl group and of a quaternary signal in the ^{13}C NMR spectrum (δ_C 167.0), corresponding to carboxylic carbon. The CI mass spectrum showed an $[M]^+$ at *m*/*z* 358, suggesting a molecular formula of $C_{22}H_{30}O_4$. The HR-ESI-MS showed an $[M+Na]^+$ at *m*/*z* 381.2052 (calculated value for $C_{22}H_{30}O_4Na$: 381.2041).

The alkaline hydrolysis of compound **2** yielded *p*-hydroxybenzoic acid, identified by TLC with an authentic standard.

In the ^{1}H NMR spectrum, some signals were very similar to those found in jaeschkeanadiol: the presence of an isopropyl group was indicated by signals at δ_H 0.82 (*d*, *J* = 6.4 Hz, 3H), 0.83 (*d*, *J* = 6.4 Hz, 3H) and 1.56 (*sept*, *J* = 6.5 Hz); another signal [δ_H 5.78 (*dd*, *J* = 10.2 and 4.1 Hz, 1H)] was attributed to H-6, which is geminal to an esterified hydroxyl group, and this was confirmed by HMBC correlation between this signal and the carboxylic carbon (δ_C 167.0) of *p*-hydroxybenzoic acid; CH_3-15 was observed as a singlet at δ_H 1.60 (3H), at a lower field than was the case for compound **1**. Two important observations account for this: firstly, the signal attributed to CH_3-11 was not a singlet, indicating that this group had one vicinal hydrogen, which meant that there could be no double bond between C-8 and C-9 in compound **2**. Secondly, there were two, instead of one, very close olefinic signals, at δ_H 5.46 (*dd*, *J* = 11.7 and 1.7 Hz, 1H) and 5.56 (*d*, *J* = 11.7 Hz, 1H), which suggested that there was a double bond between C-2 and C-3 or C-9 and C-10, these being the two locations where a double bond would leave a hydrogen atom on each carbon. As an HMBC correlation was observed between the signals for each carbon on this double bond (δ_C 134.5 and 138.2) and the hydrogen atoms of CH_3 groups 15 and 11, respectively, this double bond was assumed to be between C-9 and C-10. The β configuration of CH_3-11 was established by observing correlations of its signal with those of CH_3-15 and H-6, while both CH_3 signals 13 and 14 were correlated with H-5 in the α configuration, in the NOESY experiment. Hence, the structure of compound **2** was established as 4β-hydroxy-6α-(*p*-hydroxybenzoyloxy)dauc-9-ene (elaeochytrin B). Unlike compound **1** which was a new combination of known sesquiterpene and acyl moieties, compound **2** had a novel sesquiterpene moiety.

To our knowledge, compounds **1** and **2** have not been previously reported. The determination of their relative configuration is justified by NOESY experiments, as explained and as reported in Fig. 2. The absolute configuration of jaeschkeanadiol was determined by Sriraman et al. (1973). The stereochemistry attributed to both compounds is in accordance with what has been reported for daucane sesquiterpenoids in the genus *Ferula* (see for instance Oughlissi-Dehak et al., 2008). The presence of an anthraniloyl moiety, as described in compound **1**, is rather uncommon in natural products, although it has already been noted, for instance in saponins (Krief et al., 2005); until now it had not been known as a possible acyl moiety in sesquiterpenoids of the Apiaceae family. Isolation of saponins has been reported from a few genera of the Apiaceae family such as *Bupleurum* (Li et al., 2005), *Bupleurospermum* (Jung et al., 2007), *Centella* (Lee et al., 2006), *Eryngium* (Kartal et al., 2006), *Hydrocotyle* and *Sanicula* (Matsushita et al., 2004a,b), but we have found no previous reports of this kind of compounds in the genus *Ferula*.

All compounds isolated from *F. elaeochytris* were evaluated for their cytotoxic activity against resistant chronic myeloid leukemia

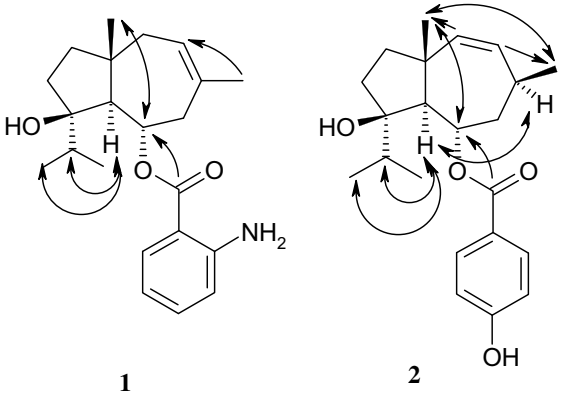

Fig. 2. Selected NOESY (↔) and HMBC (C → H) interactions in compounds **1** and **2**.

(CML) cell lines, including the dasatinib-resistant mouse cell line (DA1-3b/M2$^{BCR-ABL}$) and the imatinib-resistant human cell line (K562R). The IC_{50} of compounds that are active on at least one cell line at a concentration <100 μM are given in Table 1. All jaeschkeanadiol esters showed cytotoxic activities with IC_{50} values ranging from 7.8 to 64.1 μM. Compound **1** was the most active against DA1-3b/M2$^{BCR-ABL}$ and K562R with IC_{50} = 7.8 and 12.4 μM, respectively. Ferutinin (6-(*p*-hydroxybenzoyl)jaeschkeanadiol) was more active than teferidin (6-(benzoyl)jaeschkeanadiol) on the imatinib-resistant cell line (IC_{50} = 25.3 and 55.1 μM, respectively), while they exhibited similar cytotoxicity against the dasatinib-resistant cell line (IC_{50} = 29.1 and 29.5 μM, respectively). 6-Angeloyljaeschkeanadiol was the weakest jaeschkeanadiol ester against DA1-3b/M2$^{BCR-ABL}$ and K562R with IC_{50} = 39.2 and 64.1 μM, respectively. Among the remaining sesquiterpene esters, only compound **2** exhibited a slight cytotoxic effect against DA1-3b/M2$^{BCR-ABL}$ (IC_{50} = 65.0 μM), while it was inactive against K562R. However, 6-(*p*-hydroxybenzoyl)epoxyjaeschkeanadiol and 6-(*p*-hydroxybenzoyl)lancerotriol were inactive up to the highest tested concentration (100 μM). Taken together, these data confirm that the presence of an anthranilic moiety may be of use for cytotoxic activity, as suggested by Krief et al. (2005), although it is not absolutely necessary for jaeschkeanadiol esters, as ferutinin, teferidin and 6-angeloyljaeschkeanadiol are also cytotoxic to resistant leukemia cell lines. Conversely, the presence of the C8/C9 double bond seems to be critical, as all modifications in this bond diminish cytotoxic activity. Other, non-sesquiterpenic compounds, 5-caffeoylquinic acid, 1,5-dicaffeoylquinic acid and sandrosaponin IX were inactive on the tested cell lines. As the activity of compound **1** was thought to be promising, we then studied the cytotoxic effect of compound **1** on other leukemic or normal cell lines. On the HL60 cell line (human promyelocytic leukemia), its IC_{50} was 13.1 ± 1.0 μM (that of the positive control, camptothecin, was 400.1 ± 0.3 nM). On peripheral blood mononuclear cells (PBMCs) from healthy volunteers, it was not toxic up to 100 μM. These results demonstrate that compound **1** triggers specifically significant cytotoxicity in leukemia cell lines.

Tyrosine-kinase inhibitors (imatinib and dasatinib) are specific treatments for chronic myeloid leukemia and acute lymphoblastic leukemia bearing the Philadelphia chromosome that targets the BCR-ABL tyrosine kinase. Dasatinib is used in the case of resistance to imatinib but may itself provoke resistance in some patients (Buxeraud and Skrzypek, 2008). This and the constant need for anticancer drugs that are less toxic towards normal cells or cause fewer drug interactions explain the search for new compounds, that may be used alone, or in association with tyrosine kinase inhibitors. Compound **1** is not very potent itself, but it may be an interesting candidate for developing molecules that are active on tyrosine kinase-resistant forms of leukemia or in preventing resistance. *In vivo* investigation is required to confirm these results.

Table 1
Cytotoxicity (IC_{50} in μM[a]) of isolated jaeschkeanadiol esters against leukemia cell lines[b] *in vitro*

	K562R	DA1-3b/M2$^{BCR-ABL}$
Elaeochytrin A (1)	12.4 ± 2.6	7.8 ± 1.2
Elaeochytrin B (2)	n.a[d]	65.0 ± 1.0
Ferutinin	25.3 ± 0.5	29.1 ± 1.0
Teferidin	55.1 ± 1.0	29.5 ± 1.5
6-Angeloyljaeschkeanadiol	64.1 ± 1.0	39.2 ± 1.2
Imatinib[c]	n.a[d]	n.a[d]
Dasatinib	0.05	n.a[d]
Camptothecin[e]	2.0 ± 0.2	30.0 ± 0.5

[a] Data are mean values ± SD of three experiments.
[b] Cell lines: DA1-3b/M2$^{BCR-ABL}$ (dasatinib-resistant leukemic mouse), K562R (imatinib-resistant human chronic myeloid leukemia).
[c] IC_{50} = 0.5 μM on imatinib sensitive cell line (K562).
[d] >100 μM.
[e] IC_{50} nM, positive control.

3. Experimental

3.1. General

Mass spectra (CI-MS, ESI-MS and MALDI-TOF-MS), including HR-MS, were obtained on 55Q710 Finnigan mat, API3000 (Perkin–Elmer Sciex), Q-Tof 1 Z-spray (Waters), and Voyager DE-STR Applied Biosystems spectrometers. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 500 and 300 spectrometers and analysed with Topspin 1.3 software, in the Laboratoire d'Application de RMN (LARMN), at the same university. UV spectra were recorded on a Biochrom WPA Lightwave II UV–visible spectrometer. IR spectra were recorded on a Thermo Nicolet Avatar 320 FT-IR spectrometer. Optical rotatory power was measured on a Perkin-Elmer 343 polarimeter. MPLC was performed on a Büchi system composed of a C-605 pump module, a C-615 pump manager and a Büchi column (460 × 35 mm packed with Merck LiChroprep RP-18 25–40 μm). Silica gel 60 was provided by Merck and Sephadex LH-20 by Pharmacia. TLC were performed on Merck F_{254} silica gel plates (10 × 10 cm).

3.2. Plant material

Roots of *F. elaeochytris* were collected and authentified by Pr. Sevser Sahpaz near Yayladağı (Turkey) in 2006 (Sağıroğlu, 2005). A voucher specimen (FE 06-05) is deposited in the herbarium of the Department of Pharmacognosy and Botany, University of Lille 2, France.

3.3. Extraction and isolation

Dried and powdered roots of *F. elaeochytris* (200 g) were extracted with CH_3OH (3 × 1 l) at room temperature for 24 h. The CH_3OH extract (35.3 g) was dissolved in H_2O and submitted to liquid–liquid extraction with CH_2Cl_2 and *n*-BuOH successively to yield 22.5 and 4.8 g, respectively. Five grams of the CH_2Cl_2 extract was submitted to silicagel column chromatography eluted with a light petroleum–EtOAc gradient (90:10–20:80, v/v) to give fractions A–H. Fraction A (627 mg) was submitted to a light petroleum–EtOAc gradient (90:10–70:30, v/v), yielding angeloyljaeschkeanadiol (20 mg) and teferidin (12 mg). Fractions B (276 mg) and C (1300 mg) were purified in the same conditions to yield compound **1** (80 mg) and ferutinin (195 mg), respectively. Fraction F (463 mg), eluted with light petroleum–EtOAc (75:25, v/v), gave eight fractions (F1–F8). Fractions F3 (50 mg), F5 (49 mg) and F7 (50 mg) were separately purified on Sephadex LH-20 in CH_3OH to yield 6-(*p*-hydroxybenzoyl)epoxyjaeschkeanadiol (10 mg), 6-(*p*-hydroxybenzoyl)lancerotriol (32 mg) and compound **2** (12 mg), respectively. Two grams of *n*-BuOH extract was submitted to reverse phase medium pressure liquid chromatography (RP-MPLC), using a gradient of 10% to 100% CH_3OH in H_2O (v/v) to yield sandrosaponin IX (80 mg). Another portion of the *n*-BuOH extract (2 g) was submitted to an H_2O–CH_3OH gradient on Sephadex LH-20 to give 11 fractions (A′–K′). Fraction H′ (304 mg) was purified on Sephadex LH-20, using an H_2O/CH_3OH (92:8, v/v) mixture to yield 5-caffeoylquinic acid (chlorogenic acid) (15 mg). Fraction J′ (150 mg) was purified by Sephadex LH-20 (CH_2Cl_2/CH_3OH, 1:1, v/v) to yield 1,5-dicaffeoylquinic acid (50 mg).

3.4. Alkaline hydrolysis of *1* and *2*

Compounds **1** and **2** (5 mg each) were refluxed separately with 5% NaOH in EtOH (2 ml) for 1 h. Then the mixture was diluted with

Table 2
^{1}H (300 MHz) and ^{13}C NMR (75 MHz) data of compounds **1** and **2** in $CDCl_3$

Position	1		2	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	44.0	–	45.8	–
2	41.3	1.29 *m*	41.5	1.35 *m*
		1.65 *m*		1.60 *m*
3	31.8	1.57 *m*	30.9	1.55 *m*
		1.95 *m*		1.92 *m*
4	86.4	–	85.5	–
5	60.1	2.04 *d* (10.9)	52.3	2.45 *d* (10.5)
6	70.5	5.30 *dt* (10.2, 3.1)	70.5	5.78 *dd* (10.2, 4.1)
7	41.5	2.29 *dd* (13.2, 2.7)	46.1	2.08 *m*
		2.56 *br t* (12.3)		2.50 *m*
8	133.7	–	21.2	2.08 *d* (11.3)
9	125.4	5.56 *br t* (6.4)	138.2	5.56 *t* (11.7)
10	41.2	1.95 *m*	134.5	5.46 *dd* (11.7, 1.7)
		2.02 *m*		
11	26.7	1.83 *s*	21.3	1.24 *dd* (3.7, 2.0)
12	37.2	1.85 *m*	36.5	1.56 *sept* (6.5)
13	17.6	0.87 *d* (6.7)	17.4	0.83 *d* (6.4)
14	18.6	0.97 *d* (6.7)	18.4	0.82 *d* (6.4)
15	20.3	1.12 *s*	28.9	1.60 *s*
CO	168.4	–	167.0	–
1′	110.8	–	122.4	–
2′	151.4	–	132.0	7.89 *d* (8.6)
3′	116.3	6.68 *dd* (8.2, 1.0)	115.5	6.85 *d* (8.6)
4′	133.6	7.28 *ddd* (8.5, 7.2, 1.6)	160.8	–
5′	116.9	6.65 *ddd* (8.1, 7.1, 1.0)	115.5	6.85 *d* (8.6)
6′	131.1	7.80 *dd* (8.0, 1.6)	132.0	7.89 *d* (8.6)

H_2O (3 ml) and the EtOH was removed in vacuo. The suspension was acidified with (2 N) HCl, and then extracted with Et_2O. Anthranilic acid was identified from compound **1** with an authentic sample by TLC in light petroleum/acetone (2:1, v/v) (R_f 0.50). The spots were visualised by spraying with ninhydrin then heating. The hydrolysis of compound **2** gave *p*-hydroxybenzoic acid which was identified by direct comparison with standards using TLC in light petroleum/acetone (1.5:1, v/v) (R_f 0.63).

3.5. 6-Anthraniloyljaeschkeanadiol (elaeochytrin A, **1**)

Amorphous oil; $[\alpha]_D^{20^\circ}$ +27.6 (MeOH, *c* 0.5). For ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HR-ESI-MS *m/z* 380.2191 $[M+Na]^+$ (calcd. for $C_{22}H_{31}NO_3Na$, 380.2202); ESI-MS *m/z* = 358 $[M+H]^+$, 340, 221, 203, 138 and 120; IR ν(KBr) 3482, 3372, 2969, 2922, 1678, 1610, 1579, 1448, 1296, 1239, 1093 and 753; UV λ_{max} (MeOH) nm ($\log\varepsilon$): 217 (4.29), 249 (1.70) and 340 (0.85).

3.6. 4β-Hydroxy-6α-(p-hydroxybenzoyloxy)dauc-9-ene (elaeochytrin B, **2**)

Amorphous oil; $[\alpha]_D^{20^\circ}$ +29 (MeOH, *c* 0.5); For ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HR-ESI-MS *m/z* 381.2052 $[M+Na]^+$ (calcd. for $C_{22}H_{30}O_4Na$, 381.2041); CI-MS *m/z* = 358 $[M]^+$; IR ν(KBr) 3430, 2959, 2912, 2844, 1694, 1605, 1291 and 1166; UV λ_{max} (MeOH) nm ($\log\varepsilon$): 259 (4.1).

3.7. Biological assays

3.7.1. Cells

The human promyelocytic leukemia HL60 cell line and the Imatinib-resistant human chronic myeloid leukemia K562 cell line (K562R) have previously been described (Birnie, 1988; Mahon et al., 2000). The dasatinib-resistant leukemic mouse DA1-3b $p210^{BCR\text{-}ABL}$ cell line (DA1-3b/M2) has recently been described (Liu et al., 2008). Both K562R and DA1-3b $p210^{BCR\text{-}ABL}$ were prepared in the Institut de Recherche sur le Cancer (Lille) according to these published works. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers by Ficoll-Hypaque density sedimentation and were used as human control cells. All cells were cultured in RPMI1640 medium supplemented with 1% glutamine, 1% penicillin/streptomycin and 10% foetal calf serum (all from Gibco/BRL, Eggenstein, Germany).

3.7.2. Cytotoxicity assay

Cell toxicity was analysed by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) kit (Promega, Madison, WI, USA) according to the manufacture's protocol. Briefly, cells were seeded at a density of 10^4 cells per well in a 96-well plate and treated with drug or DMSO (vehicle). MTT (10 μl) was added to each well for the last 4 h of 48-h cultures. Absorbance was measured at 490 nm using a SpectraMaxPlus 384 spectrophotometer (Molecular Devices). All experiments were performed in triplicate. Camptothecin was used as the positive control.

References

Barthomeuf, C., Demeule, M., Grassi, J., Saidkhodjaev, A., Beliveau, R., 2006. Conferone from *Ferula schtschurowskiana* enhances vinblastine cytotoxicity in MDCK-MDR1 cells by competitively inhibiting P-glycoprotein transport. Planta Med. 72, 634–639.

Barthomeuf, C., Lim, S., Iranshahi, M., Chollet, P., 2008. Umbelliprenin from *Ferula szowitsiana* inhibits the growth of human M4Beu metastatic pigmented malignant melanoma cells through cell-cycle arrest in G1 and induction of caspase-dependent apoptosis. Phytomedicine 15, 103–111.

Birnie, G.D., 1988. The HL60 cell line: a model system for studying human myeloid cell differentiation. Br. J. Cancer Suppl. 9, 41–45.

Buxeraud, J., Skrzypek, A., 2008. Sprycel® – dasatinib. Actual. Pharm. 471, 49–52.

Chen, B., Teranishi, R., Kawazoe, K., Takaishi, Y., Honda, G., Itoh, M., Takeda, Y., Kodzhimatov, O.K., 2000. Sesquiterpenoids from *Ferula kuhistanica*. Phytochemistry 54, 717–722.

Colman-Saizarbitoria, T., Boutros, P., Amesty, A., Bahsas, A., Mathison, Y., Garrido, M.d.R., Israel, A., 2006. Ferutinin stimulates nitric oxide synthase activity in median eminence of the rat. J. Ethnopharmacol. 106, 327–332.

Eigner, D., Scholz, D., 1999. *Ferula asa-foetida* and *Curcuma longa* in traditional medical treatment and diet in Nepal. J. Ethnopharmacol. 67, 1–6.

Fraga, B.M., González, A.G., González, P., Hernandez, M.G., Larruga, C.L., 1985. Carotane sesquiterpenes from *Ferula lancerottensis*. Phytochemistry 24, 501–504.

Iranshahi, M., Kalategi, F., Rezaee, R., Shahverdi, A.R., Ito, C., Furukawa, H., Tokuda, H., Itoigawa, M., 2008. Cancer chemopreventive activity of terpenoid coumarins from *Ferula* species. Planta Med. 74, 147–150.

Jung, H.J., Nam, J.H., Park, H.J., Lee, K.T., Park, K.K., Kim, W.B., Choi, J., 2007. The MeOH extract of *Pleurospermum kamtschaticum* and its active component buddlejasaponin (IV) inhibits intrinsic and extrinsic hyperlipidemia and hypercholesterolemia in the rat. J. Ethnopharmacol. 112, 255–261.

Kartal, M., Mitaine-Offer, A.C., Paululat, T., Abu-Asaker, M., Wagner, H., Mirjolet, J.F., Guilbaud, N., Lacaille-Dubois, M.A., 2006. Triterpene saponins from *Eryngium campestre*. J. Nat. Prod. 69, 1105–1108.

Krief, S., Thoison, O., Sévenet, T., Wrangham, R.W., Lavaud, C., 2005. Triterpenoid saponin anthranilates from *Albizia grandibracteata* leaves ingested by primates in Uganda. J. Nat. Prod. 68, 897–903.

Lee, J., Jung, E., Kim, Y., Park, J., Park, J., Hong, S., Kim, J., Hyun, C., Kim, Y.S., Park, D., 2006. Asiaticoside induces human collagen I synthesis through TGFβ receptor I kinase (TβRI kinase)-independent Smad signaling. Planta Med. 72, 324–328.

Lhuillier, A., Fabre, N., Cheble, E., Oueida, F., Maurel, S., Valentin, A., Fourasté, I., Moulis, C., 2005. Daucane sesquiterpenes from *Ferula hermonis*. J. Nat. Prod. 68, 468–471.

Li, X.Q., Gao, Q.T., Chen, X.H., Bi, K.S., 2005. High performance liquid chromatographic assay of saikosaponins from Radix Bupleuri in China. Biol. Pharm. Bull. 28, 1736–1742.

Liu, J., Joha, S., Idziorek, T., Corm, S., Hetuin, D., Philippe, N., Preudhomme, C., Quesnel, B., 2008. BCR-ABL mutants spread resistance to non-mutated cells through a paracrine mechanism. Leukemia 22, 791–799.

Macho, A., Blanco-Molina, M., Spagliardi, P., Appendino, G., Bremner, P., Heinrich, M., Fiebich, B.L., Muñoz, E., 2004. Calcium ionophoretic and apoptotic effects of ferutinin in the human Jurkat T-cell line. Biochem. Pharmacol. 68, 875–883.

Mahon, F.X., Deininger, M.W., Schultheis, B., Chabrol, J., Reiffers, J., Goldman, J.M., Melo, J.V., 2000. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood 96, 1070–1079.

Maruta, Y., Kawabata, J., Niki, R., 1995. Antioxidative caffeoylquinic acid derivatives in the roots of burdock (*Arctium lappa* L.). J. Agric. Food Chem. 43, 2592–2595.

Miski, M., Ulubelen, A., Mabry, T.J., 1983. Six sesquiterpene alcohol esters from *Ferula elaeochytris*. Phytochemistry 22, 2231–2233.

Matsushita, A., Miyase, T., Noguchi, H., Vander Velde, D., 2004a. Oleanane saponins from *Sanicula elata* var. *chinensis*. J. Nat. Prod. 67, 377–383.

Matsushita, A., Sasaki, Y., Warashina, T., Miyase, T., Noguchi, H., Vander Velde, D., 2004b. Hydrocotylosides I–VII, new oleanane saponins from *Hydrocotyle sibthorpioides*. J. Nat. Prod. 67, 384–388.

Oughlissi-Dehak, K., Lawton, P., Michalet, S., Bayet, C., Darbour, N., Hadj-Mahammed, M., Badjah-Hadj-Ahmed, Y.A., Dijoux-Franca, M.-G., Guilet, D., 2008. Sesquiterpenes from aerial parts of *Ferula vesceritensis*. Phytochemistry 69, 1933–1938.

Pauli, G.F., Poetsch, F., Nahrstedt, A., 1998. Structure assignment of natural quinic acid derivatives using proton nuclear magnetic resonance techniques. Phytochem. Anal. 9, 177–185.

Poli, F., Appendino, G., Sacchetti, G., Ballero, M., Maggiano, N., Ranelletti, F.O., 2005. Antiproliferative effects of daucane esters from *Ferula communis* and *Ferula arrigonii* on human colon cancer cell lines. Phytother. Res. 19, 152–157.

Rubiolo, P., Matteodo, M., Riccio, G., Ballero, M., Christen, P., Fleury-Souverain, S., Veuthey, J.L., Bicchi, C., 2006. Analytical discrimination of poisonous and nonpoisonous chemotypes of giant fennel (*Ferula communis* L.) through their biologically active and volatile fractions. J. Agric. Food Chem. 54, 7556–7563.

Sadraei, H., Asghari, G.R., Hajhashemi, V., Kolagar, A., Hebrahimi, M., 2001. Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. Phytomedicine 8, 370–376.

Sağıroğlu, M., 2005. Türkiye *Ferula* L. (Umbelliferae) cinsi'nin revizyonu. Doktora tezi (biyoloji), Gazi Üniversitesi, Fen Bilimleri Enstitüsü, Ankara.

Sánchez-Contreras, S., Díaz-Lanza, A.M., Bernabé, M., 2000. Four new triterpenoid saponins from the roots of *Bupleurum rigidum*. J. Nat. Prod. 63, 1479–1482.

Sriraman, M.C., Nagasampagi, B.A., Pandey, R.C., Sukh, Dev, 1973. Studies in sesquiterpenes – XLIX. Sesquiterpenes from *Ferula jaeschkeana* Vatke (part 1): Jaeschkeanadiol – structure, stereochemistry. Tetrahedron 29, 985–991.

Suzuki, K., Okasaka, M., Kashiwada, Y., Takaishi, Y., Honda, G., Ito, M., Takeda, Y., Kodzhimatov, O.K., Ashurmetov, O., Sekiya, M., Ikeshiro, Y., 2007. Sesquiterpene lactones from the roots of *Ferula varia* and their cytotoxic activity. J. Nat. Prod. 70, 1915–1918.