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Sesquiterpenes from aerial parts of *Ferula vesceritensis*

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ABSTRACT

From the dichloromethane extract of aerial parts of *Ferula vesceritensis* (Apiaceae), 11 sesquiterpene derivatives were isolated. Among them five were compounds designated as 10-hydroxylancerodiol-6-anisate, 2,10-diacetyl-8-hydroxyferutriol-6-anisate, 10-hydroxylancerodiol-6-benzoate, vesceritenone and epoxy-vesceritenol. The six known compounds were identified as feselol, farnesiferol A, lapidol, 2-acetyl-jaeschkeanadiol-6-anisate, lasidiol-10-anisate and 10-oxo-jaesckeanadiol-6-anisate. All the structures were determined by extensive spectroscopic studies including 1D and 2D NMR experiments and mass spectroscopy analysis. Two of the compounds, the sesquiterpene coumarins farnesiferol A and feselol, bound to the model recombinant nucleotide-binding site of an MDR-like efflux pump from the enteropathogenic protozoan *Cryptosporidium parvum*.

1. Introduction

The *Ferula* genus (Apiaceae) comprises 130 species, which are widespread throughout central Asia, and around the Mediterranean area. Several species were used in traditional foods as well as in folk medicine as treatment for neurological disorders (tranquillizer, antihysteric), dysentery, digestive disorders, rheumatism, headache, arthritis and dizziness (Tamemoto et al., 2001). *Ferula* spp. are also known for their toxicity. A chemotype of *Ferula communis* containing a prenylated coumarin known as ferulenol and related analogues were responsible for ferulosis, a lethal haemorrhagic disease which affect domestic animals in Sardinia (Arnoldi et al., 2004). The *Ferula* genus is well documented as a good source of biologically active compounds, such as daucane esters and sesquiterpene coumarin ethers (Ghisalberti, 1994; Iranshahi et al., 2007). Its chemistry was largely studied. Daucane esters from *F. communis* and *Ferula arrigonii* showed antiproliferative activity on human colon cancer lines (Poli et al., 2005) and calcium ionophoretic and apoptotic effects in the human jurkat T-cell line (Macho et al., 2004).

Ferula vesceritensis is indigenous to Algerian Sahara (Ozenda, 1983). According to ethnobotanical investigation, fruit decoctions have been used in folk medicine to treat headaches, fever and throat infections, while the livestock avoids grazing it. A recent study carried out on the roots of *F. vesceritensis* has led to the isolation of two new sesquiterpene coumarins: 13-hydroxyfeselol and 3-angeloxycoladin, in addition to two known compounds coladin and coladonin (Ahmed et al., 2007). Ferulenol was also previously isolated from its roots (Lahouel et al., 2007). Currently, there is a considerable interest in the chemistry and pharmacology of *Ferula* species which have not extensively been studied before. In this context, we report here the investigation of the aerial parts of *F. vesceritensis* which allowed the isolation and structure elucidation of five new sesquiterpenes together with six known compounds.

Since compounds isolated from *Ferula* species were recently reported as reversal agents of multidrug resistance (MDR) in cancer cells (Barthomeuf et al., 2006), we used a parasitic model of the P-glycoprotein nucleotide-binding domain for preliminary tests to evaluate the interactions of the isolated compounds with this type of efflux pump.

Abbreviations: HECAMEG, 6-0-[(*N*-heptylcarbamoyl) methyl] α-D-glucopyranoside.

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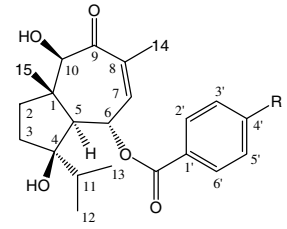
2. Results and discussion

In this work, the investigation of the dichloromethane extract from the aerial parts of *F. vesceritensis* allowed the isolation of five new sesquiterpenes **1**–**5**. Six related compounds, **6**–**11**, were identified, respectively, as feselol **6**, farnesiferol A **7** (Shahverdi et al., 2005), 2-acetyl-jaechkeanadiol-6-anisate **8** (Al-Yahya et al., 1998), lasidiol-10-anisate **9** (Cumanda et al., 1991), 10-oxojaeschkeanadiol-6-anisate **10** (Arnoldi et al., 2004) and lapidol **11** (Gonzalez et al., 1993). The structures of the known compounds were elucidated by analysis of their spectral data (including 2D NMR and MS studies) and by comparison with spectroscopic data previously reported.



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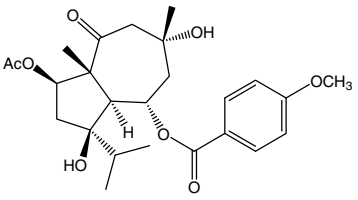
2, R = H



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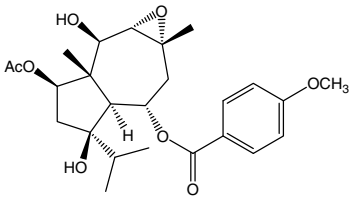

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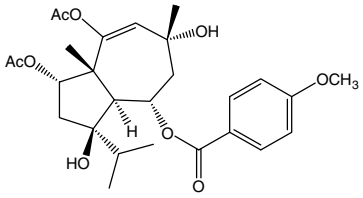

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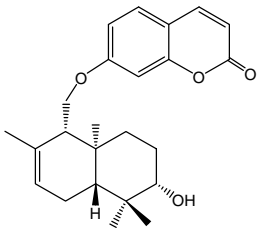

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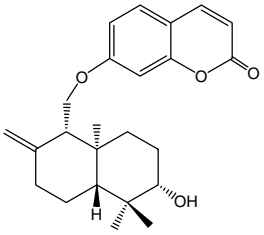

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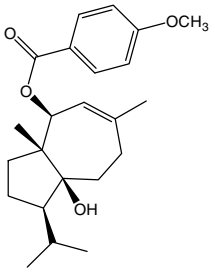

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HRCI/MS revealed a molecular formula of $C_{23}H_{30}O_6$ for compound **1**. Examination of the NMR data of **1** indicated unambiguously the presence of an anisate moiety with characteristic aromatic protons at δ_H 8.01 (2H, *d*, 8.8 Hz), 6.96 (2H, *d*, 8.8 Hz), and methoxy protons at δ_H 3.89 (*s*). The ^{13}C NMR spectrum of **1** displayed 21 signals beside those of the *para*-anisate moiety (δ_C 165.9, 121.9, 131.9, 113.9 and 163.9), 15 resonances could be assigned to a sesquiterpene sub-structure. Analysis of ^1H NMR data revealed the presence of an isopropyl moiety [δ_H 0.98 (3H, *d*, 6.7 Hz), 0.86 (3H, *d*, 6.7 Hz) and 1.91 (1H, *m*)] in addition to two methyl groups [δ_H 1.07 (3H, *s*), 1.96 (3H, *s*)]. These structural elements suggested for compound **1** a carotane skeleton corresponding to a bicyclic structure with a condensed five and seven membered ring system, previously isolated from different *Ferula* species (Ahmed et al., 2001). Long-range ^1H–^{13}C correlations associating the methyl group (δ_H 1.07) to the carbon signals at 37.8 (C-2), 48.7 (C-1), 53.8 (C-5) and 85.0 ppm (C-10), led to the attribution of the methyl group to the C-15 position. Two saturated methylene groups, with a vicinal relationship deduced from the COSY spectrum, were identified by HSQC analysis [δ_H 1.60 (1H, *m*), 2.06 (1H, *m*) and δ_C 37.8] and [δ_H 1.92 (1H, *m*), 1.70 (1H, *m*) and δ_C 31.8] and deduced from HMBC information, they were located at positions C-2 and C-3. HMBC correlations observed for the isopropyl moiety with the carbon at 86.6 ppm indicated the presence of a hydroxyl group at the C-4 position and achieved attribution of the cyclopentane ring pattern. The remaining proton signals, an olefinic proton (δ_H 6.23, *s*), an olefinic methyl (δ_H 1.96, 3H, *s*), and two oxygenated methine protons [δ_H 4.08 (1H, *d*, 3.0 Hz), 6.11 (1H, *d*, 11.0 Hz)] assigned by HSQC experiments, respectively, to δ_C 141.3 (C-7), 21.6 (C-14), 85.0 (C-10) and 71.9 (C-6) were associated with the cycloheptane ring. The substitution pattern of this ring was deduced by analysis of long-range ^1H–^{13}C couplings, (i) between the hydroxyl group at δ_H 4.18 (10-OH) and the carbons at δ_C 48.7 (C-1), 85.0 (C-10) and the carbonyl carbon at 201.8 (C-9) and (ii) those associating the methyl protons at δ_H 1.96 (14-CH_3) with the carbons at δ_C 132.3 (C-8), 141.3 (C-7) and the carbonyl carbon at 201.8 (C-9). Finally, the location of the anisate moiety was deduced by the correlation between the proton at δ_H 6.11 (H-6) and the ester function [δ_C 165.9 (–CO–O)] of the anisate group. Relative stereochemistry of **1** was then established unambiguously by analysis of its NOESY spectrum. In particular, spatial correlations were observed between 15-CH_3 and H-6 for one side of the molecule and between H-10, isopropyl proton signals, anisate proton signals and H-5 for the other side. Except for the substitution of the 10-position, the structure of compound **1** was then closely related to lancerodiol-6-anisate previously reported in *Ferula lancerottensis* (Fraga et al., 1985) and *Ferula linkii* (Diaz et al., 1986), and was thus identified as 10-hydroxylancerodiol-6-anisate.

Compound **2** was related to the molecular formula of $C_{22}H_{28}O_5$ by HRCI/MS. Its ^1H NMR spectrum was closely similar to that of compound **1**, except for the lack of signal corresponding to a methoxy group and the modification of the spin system of the aromatic moiety suggesting here a non-substituted aryl group. The difference of 30 u.m.a. observed between both mass spectra corroborated the loss of a methoxy group between structures. In

agreement with analysis of spectral data of **2** including NOESY information, this compound was identified as 10-hydroxylancerodiol-6-benzoate.

The molecular formula $C_{25}H_{34}O_8$ for compound **3** was determined by HRCI/MS. Analysis of its ^{1}H NMR and ^{13}C NMR spectra indicated that **3** presented a carotane skeleton, with an isopropyl group and two methyl groups, esterified by an anisate group at C-6 position. Long-range couplings deduced from the HMBC between methyl protons at δ_H 1.38 (15-CH_3) and carbons at δ_C 48.3 (C-5), 59.3 (C-1), 82.4 (C-2) and 212.2 (C-10) were indicative of some substitution pattern modifications of the terpenic skeleton, when compared to those of compounds **1** and **2**. Considering the cycloheptane ring, the methyl protons at δ_H 1.34 (14-CH_3, *s*) showed HMBC correlations with carbon resonances associated with an oxygenated quaternary carbon (δ_C 71.4, C-8), and two saturated methylenes (δ_C 43.9, C-7), and (δ_C 57.8, C-9). The exact location of the saturated methylene groups were deduced from HMBC data which linked on one hand, H-9 (δ_H 2.82, 3.55 and δ_C 57.8) to the carbonyl carbon (δ_C 212.2, C-10) and on the other hand, H-7 (δ_H 1.90, 2.14 and δ_C 43.9) to the saturated methine carbon (δ_C 48.3, C-5). The last information for the substitution pattern of the cycloheptane ring concerned the anisate moiety. Its exact location was deduced from long-range correlation between the proton at δ_H 5.80 (*m*, H-6) and the ester function [δ_C 166.4 (–CO–O)] of the anisate group. Finally, the presence of an acetoxyl group at C-2 was supported by HMBC correlations of methyl protons at δ_H 1.97 (3H, *s*) and saturated methine proton at δ_H 4.94 (H-2) with the same signal at δ_C 169.4 assigned to a carbonyl carbon. The relative stereochemistry of **3** was determined by analysis of the NOESY spectrum which showed spatial relationships between H-2, H-3α, H-5, H-7α, H-9α, H-11 and H-13 for one side of the molecule and between H-3β, H-6, H-7β, H-9β, 14-CH_3, 15-CH_3, and acetyl protons for the other side of the molecule. Compound **3** which presented an original substitution pattern of the sesquiterpene skeleton was named vesceritenone.

Compound **4** had the same molecular formula as **3** ($C_{25}H_{34}O_8$) deduced from HRCI/MS. Analysis of NMR data showed that it belonged to the same group of sesquiterpenes as the previous compounds. Furthermore, as for compound **3**, an anisate group at the C-6 position and an acetoxyl group at the C-2 position were also identified in compound **4**. Nevertheless, on the basis of NMR data and HMBC correlations, some differences were observed between both compounds. NMR data of **4** indicated the presence of two additional oxygenated methines [δ_H 2.88 (*d*, 5.0 Hz) and δ_C 66.7, and δ_H 3.69 (*d*, 5.0 Hz) and δ_C 74.6]. Moreover, compared to compound **3**, no ketone as well as no additional unsaturated carbons was detected in compound **4**, suggesting then the presence of a supplementary ring such as an epoxy function. The exact location of the oxygenated methine groups was deduced from HMBC data which linked on one hand, the methyl protons signal 14-CH_3 (δ_H 1.51) to the first oxygenated methine carbon (δ_C 66.7, C-9) and on the other hand the methyl protons signal 15-CH_3 (δ_H 1.33) to the second oxygenated methine carbon (δ_C 74.6, C-10). By analysis of the NOESY spectrum of **4**, major spatial correlations were observed between 15-CH_3, H-9 and 14-CH_3 for one side of the molecule, and between H-10 and H-5 for the other side. Relative stereochemistry of the oxygenated carbons C-8, C-9 and C-10 implicated then the presence of the epoxy function at the C-8/C-9 position and with an α-orientation. According to the literature, several daucane sesquiterpenes were previously reported in the genus *Ferula* and spectral data of **4** were in agreement with those published (Chen et al., 2000; Fraga et al., 1985). Compound **4** which presented an original substitution pattern of the sesquiterpene skeleton was named epoxyvesceritenol.

To compound **5** was assigned a molecular formula of $C_{27}H_{36}O_9$ by HRCI/MS. Analysis of its ^{1}H NMR and ^{13}C NMR spectra indicated that **5** presented a similar carotane skeleton to those of the previous compounds. Furthermore, it was also esterified by an anisate group at C-6 and by an acetoxyl group at C-2. Long-range couplings deduced from the HMBC spectrum between the methyl protons at δ_H 1.36 (15-CH_3, *s*) and carbons at δ_C 49.2 (C-5), 52.8 (C-1), 79.6 (C-2), 153.2 (C-10), revealed the presence of an ethylenic oxygenated carbon at C-10. Considering the cycloheptane ring, the methyl protons at δ_H 1.61 (14-CH_3, *s*) showed long-range HMBC correlations with an oxygenated quaternary carbon (δ_C 82.2, C-8), a saturated methylene (δ_C 40.6, C-7), and a second ethylenic carbon (δ_C 123.9, C-9). These assignments were corroborated by the HMBC correlations observed between the olefinic proton signal H-9 (δ_H 5.48, *s*) and carbons at δ_C 52.8 (C-1), 153.2 (C-10), 82.2 (C-8), 40.6 (C-7), 24.3 (C-14). The presence of two additional methyl signals at δ_H 2.11 (3H, *s*) and 2.03 (3H, *s*) which showed HMBC correlations, respectively, with carbons at δ_C 169.4 and 169.9 were assigned to acetoxyl groups. Their linkages were determined through HMBC correlations between on one hand the signal at δ_H 5.12 (H-2, *d*, 5.5 Hz) and the carbonyl carbon at δ_C 169.9, and on the other hand, the long-range coupling between the methyl group at δ_H 2.11 and C-10 (δ_C 153.2). The linkage of the latter acetoxyl group to C-10 was confirmed by NOESY effects observed between H-2 (δ_H 5.12) and both acetoxyl groups δ_H 2.11 and 2.03. On basis of the COSY experiment, neighbouring relationships were established between, on one hand, a proton at δ_H 5.12 (H-2, *d*, 5.5 Hz) and the saturated methylene group [δ_H 1.92 (H-3, *m*), 2.04 (H-3, *m*) and δ_C 38.7] and, on the other hand, a proton at

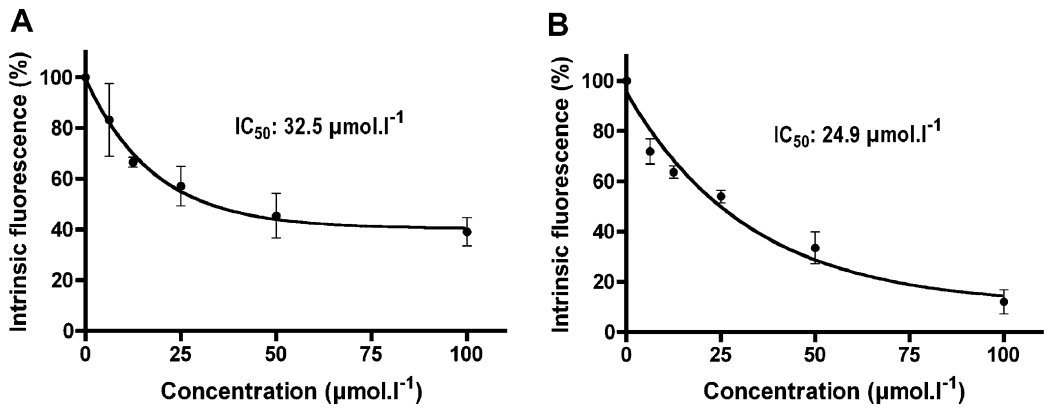

Fig. 1. Dose-dependent binding of the sesquiterpene coumarins **6** and **7** to recombinant *C. parvum* H6-NBD1. The quenching of the intrinsic fluorescence of the recombinant H6-NBD1 by **6** (A) and **7** (B) was measured at 328 nm. The values are the mean ± SD of three separate experiments.

δ_H 5.94 (H-6, *m*) and the saturated methine group [δ_H 2.97 (H-5, *d*, 11.0 Hz) and δ_C 49.2]. The long-range HMBC correlations of the proton signal at δ_H 2.97 with the carbons at [δ_C 69.1 (C-6), 84.9 (C-4), 40.6 (C-7), 153.2 (C-10), 36.4 (C-11) and 18.4 (C-15)] supported the structure of **5**. The relative stereochemistry of **5** was determined by analysis of the NOESY spectrum which showed spatial relationships between H-2, H-3β, H-6, H-7β, 14-CH_3, and 15-CH_3 for one side of the molecule and between H-3α, H-11, H-5 and H-2′ for the other side of the molecule, indicating that the anisate group, the two acetyl groups and the isopropyl group possessed the same orientation. The comparison of the data to those reported for ferutriol (Diaz et al., 1986) and kuhistanicaol G (Chen et al., 2000) allowed the assignment of compound **5** as 2,10-diacetyl-8-hydroxyferutriol-6-anisate. This study of a Sahara *Ferula* led to the identification of five new daucane sesquiterpenes among which, two compounds possessed original substitution patterns of the cycloheptane ring. It is also important to mention that our investigation carried out on aerial parts afforded nine sesquiterpene daucanes and two sesquiterpene coumarins, while the chemical investigation of the roots (Ahmed et al., 2007) related only the presence of four sesquiterpene coumarins. Furthermore, as related for many *Ferula,* the accumulation of the sesquiterpene coumarins might be in the roots. In addition, the known sesquiterpene lasidiol-10-anisate **9**, described here, had not been reported in *Ferula* genus previously. It was only reported in another Asteraceae species, *Xanthium catharticum* (Cumanda et al., 1991).

In our course to identify natural efflux pump inhibitors and in order to see if some of these compounds could be potential modulators of multidrug resistance, preliminary tests were performed on a parasitic model receptor related to the P-glycoprotein. These proteins are involved in the active efflux of xenobiotics and responsible for the resistance of cancer cells and some parasites to chemotherapy (Sharom et al., 1999; Klokouzas et al., 2003). When assayed on the recombinant nucleotide-binding domain of CpABC3, a MDR-like transporter from the protozoan parasite *Cryptosporidium parvum* (Lawton et al., 2007), only the sesquiterpene coumarins **6** and **7** exhibited a dose-dependent affinity towards the recombinant H6-NBD1 (Fig. 1). In addition, their structure is similar to conferone, a sesquiterpene isolated from another member of the genus, *F. schtschurowskiana* which was also very efficient against the P-glycoprotein from MDCK cells membranes (Barthomeuf et al., 2006). The inhibition curves are similar to those reported for conferone using a different test (Barthomeuf et al., 2006), but the IC_{50} reported here are higher and comparable to the competitive inhibitor TNP-ATP (Lawton et al., 2007). However, no inhibition of the ATPase activity of the recombinant domain was evidenced (data not shown). These results are consistent with a binding of the compounds to another or many other sites on H6-NBD1, including the reported vicinal steroid binding site (Dayan et al., 1997; Conseil et al., 1998), rather than to the catalytic pocket. Finally, the binding of the two new sesquiterpene coumarins described in this study to the nucleotide-binding domain of our MDR-like transporter model confirms the chemotherapeutic potential of these compounds as reversing agents of these efflux pumps.

3. Experimental

3.1. Plant material

Aerial parts of *F. vesceritensis* Coss et Dur ex Batt were collected in May 2005 from Ghardaïa area, located in Algerian Sahara. The plant was identified by M. Ouled Belkheir and Dr. A. Chehma from Department of Biological Sciences of Ouargla University. A voucher specimen has been deposited in the Department of Chemistry, Constantine University (AM#112).

3.2. General experimental procedures

NMR spectra were recorded on DRX 500 spectrometer (500 MHz for ^1H) with $CDCl_3$ as solvent (internal reference, TMS). ESIMS were recorded with a Thermo LCQ Advantage, ion-trap spectrometer while HRCI/MS and HRESI/MS were recorded with Thermo Finnigan Mat 95XL. TLC was carried out using Merck silica gel Si 60 F_{254} 20 × 20 cm plastic and aluminium sheets and RP-18 F_{254S} 20 × 20 cm aluminium sheets. Analytical HPLC was carried out on a Thermo Separation Products system equipped with a P-4000 quaternary gradient pump system, a UV-6000LP photodiode array detector using analytical Zorbax XDB C8 (250 × 4.6 ID mm; 5 μm; 100 Å), the solvent system was: TFA 0.01% in water (solvent A) and TFA 0.01% in acetonitrile (solvent B), starting with 45% for 5 min then a linear gradient from 45% to 90% in 40 min. The flow rate was 1 ml/min; the PDA detection range was 200–400 nm. Semipreparative HPLC was performed on a 2 millipore Waters Model 510 pumps, with an automated gradient controller and Waters 490 programmable multiwavelength detector using Purospher STAR RP-18 5 μm (100 × 25 mm) column with methanol–water gradient. MPLC were carried out using Merck silicagel 60 (40–63 μm), Lichroprep 100 DIOL (40–63 μm), and Lichroprep 60 RP-18 (40–63 μm) with UV detection at 254 and 366 nm.

Table 1
^1H-NMR spectral data for **1**–**5** [500 MHz, $CDCl_3$, δ_H/ppm, mult. (*J*/Hz)]

Position	1	2	3	4	5
2	1.60 *m* (α)	1.66 *m* (α)	4.94 *d* (5.7)	5.06 *d* (5.7)	5.12 *d* (5.5)
	2.06 *m* (β)	1.91 *m* (β)			
3	1.70 *m* (β)	1.64 *m* (β)	2.05 *m* (α)	1.95 *m* (α)	1.92 *m* (α)
	1.92 *m* (α)	2.06 *m* (α)	2.16 *m* (β)	2.09 *m* (β)	2.04 *m* (β)
4	–	–	–	–	–
5	2.65 *d* (11.0)	2.68 *d* (11.0)	3.69 *d* (10.1)	2.43 *d* (10.7)	2.97 *d* (11.0)
6	6.11 *d* (11.0)	6.15 *d* (11.0)	5.80 *m*	5.46 *t* (10.7)	5.94 *m*
7	6.23 *s*	6.23 *s*	1.90 *m* (β)	1.82 *d* (12.0)	2.10 *m*
			2.14 *m* (α)	2.30 *d* (12.0)	2.89 *d* (5.0)
9	–	–	2.82 *d* (12.9) (β)	2.88 *d* (5.0)	5.48 *s*
			3.55 *d* (12.9) (α)		
10	4.08 *d* (3.0)	4.09 *d* (3.0)	–	3.69 *d* (5.0)	–
11	1.91 *m*	1.92 *m*	1.64 *m*	1.97 *m*	1.62 *m*
12	0.86 *d* (6.7)	0.85 *d* (6.7)	0.86 *d* (6.7)	0.83 *d* (6.7)	0.83 *d* (6.7)
13	0.98 *d* (6.7)	0.99 *d* (6.7)	0.95 *d* (6.7)	0.95 *d* (6.7)	0.97 *d* (6.7)
14	1.96 *s*	1.97 *s*	1.34 *s*	1.51 *s*	1.61 *s*
15	1.07 *s*	1.07 *s*	1.38 *s*	1.33 *s*	1.36 *s*
2′	8.01 *d* (8.8)	8.07 *t* (7.6)	6.96 *d* (8.8)	7.95 *d* (8.5)	7.97 *d* (8.5)
3′	6.96 *d* (8.8)	7.49 *t* (7.6)	7.99 *d* (8.8)	6.95 *d* (8.5)	6.95 *d* (8.5)
4′	–	7.61 *t* (7.6)	–	–	–
5′	6.96 *d* (8.8)	7.49 *t* (7.6)	7.99 *d* (8.8)	6.95 *d* (8.5)	6.95 *d* (8.5)
6′	8.01 *d* (8.8)	8.07 *t* (7.6)	6.96 *d* (8.8)	7.95 *d* (8.5)	7.97 *d* (8.5)
Ar-OCH_3	3.89 *s*	–	3.89 *s*	3.88 *s*	3.88 *s*
2-OCO-CH_3	–	–	1.97 *s*	–	2.03 *s*
8-O*H*	4.18 *d* (3.0)	4.19 *d* (3.0)	–	–	–
10-OCO-CH_3	–	–	–	–	2.11 *s*

Table 2
^{13}C NMR spectral data for compounds **1–5** (500 MHz, $CDCl_3$, δ_C/ppm)

Position	1	2	3	4	5
1	48.7	48.7	59.3	53.2	52.8
2	37.8	37.9	82.4	79.7	79.6
3	31.8	32.0	40.6	39.2	38.7
4	86.6	86.5	83.6	85.2	84.9
5	53.8	53.7	48.3	54.1	49.2
6	71.9	72.3	70.6	69.0	69.1
7	141.3	141.0	43.9	44.1	40.6
8	132.3	132.5	71.4	55.2	82.2
9	201.8	201.8	57.8	66.7	123.9
10	85.0	85.0	212.2	74.6	153.2
11	37.0	37.0	36.4	36.9	36.4
12	13.3	17.2	17.4	17.4	17.5
13	18.3	18.3	18.3	18.1	18.2
14	21.6	21.6	33.4	23.0	24.3
15	14.4	14.4	17.5	13.6	18.4
O-*CO*-Ar	165.9	166.1	166.4	166.1	166.8
1′	121.9	129.7	121.8	122.1	122.2
2′	131.9	129.8	131.8	131.7	131.8
3′	113.9	128.7	114.1	114.1	113.9
4′	163.9	133.6	164.1	163.8	164.2
5′	113.9	128.7	114.1	114.1	113.9
6′	131.9	129.8	131.8	131.7	131.8
Ar-O*CH*$_3$	55.5	–	55.6	55.5	55.5
2-O*CO*-CH_3	–	–	169.4	170.9	169.9
2-OCO-*CH*$_3$	–	–	21.0	21.3	21.2
10-O*CO*-CH_3	–	–	–	–	169.4
10-OCO-*CH*$_3$	–	–	–	–	22.1

3.3. Extraction and isolation

Air-dried and coarsely powdered (824 g) aerial parts were extracted with hexane, dichloromethane and methanol (2.5 l × 3, each) at room temperature. The second extract, gave 16.5 g of dark green viscous mass; 15.7 g of it was fractioned by MPLC (Si gel, 40–63 μm, column 460 × 36 mm, 7 ml/min) using a gradient of 0:100% ethyl acetate–hexane to afford 16 fractions.

Fraction F6 (400 mg) which was eluted with 10% EtOAc in hexane was separated by MPLC (Si gel, 40–63 μm, column 230 × 26 mm, 7 ml/min) using a gradient of EtOAc in hexane from 0% to 10% to obtain compound **1** (8.7 mg) and compound **2** (11.0 mg).

Fraction F7 (500 mg) which was eluted with 20% EtOAc in hexane was purified by MPLC in the same conditions as fraction F6 to give compounds **4** (4.4 mg), **5** (6.8 mg) and **11** (5.3 mg).

Fraction F12 (2.2 g), which was eluted with 30% EtOAc in hexane, was again separated by MPLC (Si gel RP-18 40–63 μm, column 460 × 26 mm, 7 ml/min) using a gradient of 30–100% MeOH in H_2O. We obtained compound **8** (80.3 mg), and several subfractions, particularly subfraction 3 [MeOH/H_2O (4:6), 52 mg], subfraction 6 [MeOH/H_2O (6:4), 67 mg], subfraction 9 [MeOH/H_2O (7:3), 34 mg], and subfraction 19 [MeOH/H_2O (85:15), 95 mg]. Preparative TLC (Si gel, chloroform–ethyl acetate (90:10, 85:15) of subfractions 3 and 6 were used for further purification leading to compounds **10** (6.0 mg) and **3** (5.4 mg), respectively. Subfraction 9 was further separated by prep. TLC [Si gel, toluene–hexane (80:20)] and then purified on a Sephadex (LH-20) column using chloroform–acetone (70:30) to give compound **9** (6.5 mg). Subfraction 19 was purified on semi-preparative HPLC using a gradient of MeOH in H_2O (80–100%) to afford compounds **6** (25.2 mg) and **7** (15.3 mg).

3.4. Affinity towards the recombinant C. parvum H6-NBD1

The binding of the compounds was evaluated with a purified recombinant nucleotide-binding domain of the CpABC3 from the enteropathogenic protozoan parasite *C. parvum* (Lawton et al., 2007). Fluorescence experiments were performed in triplicate on a Perkin–Elmer LS-3B spectrofluorimeter in a 1 cm-path quartz microcuvette. The recombinant protein was diluted at a concentration of 0.25 μM in 390 μl of 50 mM Tris pH 7.3, 100 mM KCl, 0.02% HECAMEG. The ligands were added in 10 μl and the mixture was incubated for 30 min at 20 ± 2 °C. Intrinsic fluorescence emission at 328 nm was measured upon excitation at 295 nm. Controls were made in the same conditions with the last dialysis buffer instead of the H6-NBD1, to eliminate any interference due to the imidazole used during the purification process. The compounds were dissolved as 40 mM stock solutions in DMSO and serially diluted in the assay buffer with a final solvent concentration of 0.25%. Controls contained the same DMSO concentration. Statistics and curve fitting were made with the Prism 4 software program from GraphPad (San Diego, CA).

3.5. 10-Hydroxylancerodiol-6-anisate (*1*)

Amorphous white powder; $[\alpha]_D^{20}$ +81.7 (c 0.08, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ε) 259.5 (4.30). For ^1H NMR and ^{13}C NMR data see Tables 1 and 2. HRCI/MS $[M+H]^+$ *m/z* 403.21195 (calc. for $C_{23}H_{30}O_6$ 403.2121). ESI/MS $[M+Na]^+$ *m/z* 425, $[2M+Na]^+$ *m/z* 827.2.

3.6. 10-Hydroxylancerodiol 6-benzoate (*2*)

Amorphous white powder; $[\alpha]_D^{20}$ +87.7 (c 0.075, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ε) 232 (3.92). For ^1H NMR and ^{13}C NMR data see Tables 1 and 2. HRCI/MS $[M+H]^+$ *m/z* 373.20137 (calc. for $C_{22}H_{28}O_5$ 373.2015). ESI/MS $[M+Na]^+$ *m/z* 395.2.

3.7. Vesceritenone (*3*)

Amorphous white powder; $[\alpha]_D^{25}$ −239.2° (CH_2Cl_2; c 0.125); UV (MeOH) λ_{max} nm (log ε) 257 (3.78). For ^1H NMR and ^{13}C NMR data see Tables 1 and 2. HRCIMS $[M+H]^+$ *m/z* 463.23315 (calc. for $C_{25}H_{34}O_8$ 463.2332). ESI/MS $[M+Na]^+$ *m/z* 485.2, $[2M+Na]^+$ *m/z* 947.1.

3.8. Epoxyvesceritenol (*4*)

Amorphous white powder; $[\alpha]_D^{20}$ +27.5 (c 0.17, CH_2Cl_2); UV (MeOH) λ_{max} nm (logε) 258 (4.23). For ^1H NMR and ^{13}C NMR data see Tables 1 and 2. HRCI/MS $[M+H]^+$ *m/z* 463.23303 (calc. for $C_{25}H_{34}O_8$ 463.2332). ESI/MS $[M+Na]^+$ *m/z* 485.1, $[2M+Na]^+$ *m/z* 947.1.

3.9. 2,10-Diacetyl-8-hydroxyferutriol-6-anisate (*5*)

Amorphous white powder; $[\alpha]_D^{20}$ +13.68 (c 0.24, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ε) 257 (3.82). For ^1H NMR and ^{13}C NMR data see Tables 1 and 2. HRMS-ESI $[M+Na]^+$ *m/z* 527.22603 (calc. for $C_{25}H_{34}O_8Na$ 527.2257). ESI-MS $[M+Na]^+$ *m/z* 527.2, $[M+K]^+$ *m/z* 543.1.

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References

Ahmed, A.A., Hegazy, M.-E.F., Zellagui, A., Rhouati, S., Mohamed, T.A., Sayed, A.A., Abdella, M.A., Ohta, S., Hirata, T., 2007. Ferulsinaic acid, a sesquiterpene coumarin with a rare carbon skeleton from *Ferula* species. Phytochemistry 68, 680–686.

Ahmed, A.A., Abd El-Razek, M., Nassar, M.I., Izumi, S., Ohta, S., Hirata, T., 2001. An eudesmanolide and carotene from *Ferula sinaica*. Phytochemistry 57, 513–515.

Al-Yahya, M.A., Muhammad, I., Mirza, H.H., 1998. Antibacterial constituents from the rhizomes of *Ferula communis*. Phytother. Res. 12, 335–339.

Arnoldi, L., Ballero, M., Fuzzati, N., Maxia, A., Mercalli, E., Pagni, L., 2004. HPLC-DAD-MS identification of bioactive secondary metabolites from *Ferula communis* roots. Fitoterapia 75, 342–354.

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.

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.

Conseil, G., Baubichon-Cortay, H., Dayan, G., Jault, J.M., Barron, D., Di Pietro, A., 1998. Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP and steroid-binding sites on mouse P-glycoprotein. Proc. Natl. Acad. Sci. USA 95, 9831–9836.

Cumanda, J., Marinoni, G., De Bernardi, M., Vidari, G., Vita Finzi, P., 1991. New sesquiterpenes from *Xanthium catharticum*. J. Nat. Prod. 54, 460–465.

Dayan, G., Jault, J.M., Baubichon-Cortay, H., Baggetto, L.G., Renoir, J.M., Beaulieu, E.E., Gros, P., Di Pietro, A., 1997. Binding of steroid modulators to recombinant cytosolic domain from mouse P-glycoprotein in close proximity to the ATP site. Biochemistry 36, 15208–15215.

Diaz, J.G., Fraga, B.M., Gonzalez, A.G., Hermandez, M.G., Perales, A., 1986. Carotane sesquiterpenes from *Ferula linkii*. Phytochemistry 25, 1661–1665.

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

Ghisalberti, E.L., 1994. The daucanes class of sesquiterpenes. Phytochemistry 37, 597–623.

Gonzalez, A.G., Diaz, J.G., Lopez, L.A., Valencia, E., De Paz, P.P., Barrera, J.B., 1993. Sesquiterpene esters and sesquiterpene coumarin ethers from *Ferula linkii*. Phytochemistry 33, 863–866.

Iranshahi, M., Arfa, P., Ramezani, M., Jaafari, M.R., Sadeghian, H., Bassarello, C., Piacente, S., Pizza, C., 2007. Sesquiterpene coumarins from *Ferula szowitsiana* and *in vitro* antileishmanial activity of 7-prenyloxycoumarins against promastigotes. Phytochemistry 68, 554–561.

Klokouzas, A., Shahi, S., Hladky, S.B., Barrand, M.A., Van Veen, H.W., 2003. ABC transporters and drug resistance in parasitic protozoa. Int. J. Antimicrob. Agents 22, 301–317.

Lahouel, M., Zini, R., Zellagui, A., Rhouati, S., Carrupt, P.-A., Morin, D., 2007. Ferulenol specifically inhibits succinate ubiquinone reductase at the level of the ubiquinone cycle. Biochem. Biophys. Res. Commun. 355, 252–255.

Lawton, P., Pélandakis, M., Pétavy, A.F., Walchshofer, N., 2007. Overexpression, purification and characterization of a hexahistidine-tagged recombinant extended nucleotide-binding domain 1 (NBD1) of the *Cryptosporidium parvum* CpABC3 for rational drug design. Mol. Biochem. Parasitol. 152, 101–107.

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

Ozenda, P., 1983. Flore du Sahara, second ed. Centre National de la Recherche Scientifique (CNRS), Paris. 622p., p. 359.

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

Shahverdi, A.R., Mirjani, R., Amin, G., Shafiee, A., Iranshahi, M., 2005. Bleaching of Serratia by some coumarins: a spectrometric study. J. Basic Microbiol. 45, 470–474.

Sharom, F.J., Liu, R., Romsicki, Y., Lu, P., 1999. Insights into the structure and substrate interactions of the P-glycoprotein multidrug transporter from spectroscopic studies. Biochim. Biophys. Acta 1461, 327–345.

Tamemoto, K., Takaishi, Y., Chen, B., Kawazoe, K., Shibata, H., Higuti, T., Honda, G., Ito, M., Takeda, Y., Kodzhimatov, O.K., Ashurmetov, O., 2001. Sesquiterpenoids from the fruits of *Ferula kuhistanica* and antibacterial activity of the constituents of *F. kuhistanica*. Phytochemistry 58, 763–767.