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Short communication

Anti-proliferative activity of aguerin B and a new rare *nor*-guaianolide lactone isolated from the aerial parts of *Centaurea deflexa*

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ABSTRACT

The phytochemical investigation of the aerial parts of *Centaurea deflexa* led to the identification of 21 compounds, among which three phenolic acids, one sterol, ten flavonoids, one phenylpropanoid derivative, two lignans and four sesquiterpene lactones. One of the latter compounds was a new, rare active principle (**1**) having an uncommon 15-*nor*-guaianolide skeleton. The biological investigation was carried out through a bio-guided assay fractionation of *C. deflexa* extracts and highlighted an anti-proliferative activity of two sesquiterpene lactones, aguerin B and the newly identified 15-*nor*-guaianolide (**1**) against human pancreatic and colonic cancer cells. Of the two compounds, only aguerin B showed to induce apoptotic cell death, confirming the role as pro-apoptotic moiety of the α-methylene-γ-lactone ring present in aguerin B but not in **1**.

1. Introduction

Centaurea deflexa Wagenitz (Sect. Cheirolepis) (Syn: *Phaeopappus declinatus* Boiss., *Centaurea nivea* (Bornm.) Wagenitz. var. *declinata* (Boiss.) Wagenitz) is a perennial plant belonging to the Asteraceae family that abundantly grows in Turkey and, although its use in Turkish folk medicine is not well described, many other species of the same genus (i.e. *Centaurea cyanus* and *Centaurea scabiosa*) are widely used against cough and itch, as liver-strengthening and ophthalmic remedies [1]. In addition, *Centaurea calcitrapa*, *Centaurea solstitialis* and *Centaurea melitensis* are used for their hypoglycemic effects, *C. calcitrapa*, *Centaurea iberica* and *Centaurea jacea* for their antipyretic activity [2] and other *Centaurea* species for their antioxidant [3] and anti-inflammatory properties [4]; *in vitro* cytotoxic activity against different human cancer cell lines has been also reported for several *Centaurea* species [5]. The major constituents identified in *Centaurea* L. species, responsible for most of their pharmacological properties are sesquiterpene lactones and flavonoids, even though unsaturated hydrocarbons have been recently identified [6,7]. In the present study, we investigated for the first time the phytochemical composition and the biological activity of *C. deflexa*, identifying a new, rare *nor*-guaianolide lactone that exhibited an anti-proliferative activity against human cancer cell lines as well as aguerin B, another sesquiterpene lactone isolated from this plant. These two compounds, although belonging to the same chemical family (guaianolide lactones), showed different potency in anti-proliferative activity and behaved differently in regard with apoptosis induction, since only aguerin B exhibited a pro-apoptotic activity. This evidence is in keeping with the presence of the α-methylene-γ-lactone ring in aguerin B but not in *nor*-guaianolide, which has been previously reported to play an important role in cytotoxic and pro-apoptotic activity of sesquiterpene lactones, by reacting through Michael-type addition with biological nucleophiles as thiol residues [8].

2. Results and discussion

The phytochemical investigation of *C. deflexa* led to the identification and chemical characterization of 21 compounds, belonging to various classes of compounds: three phenolic acids, a sterol, ten flavonoids (three of which as C-glycosides), a phenylpropanoid

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derivative, two lignans and four sesquiterpene lactones (Fig. E1 Supplementary Information), one of which was a new natural compound with a rare 15-*nor*-guaianolide skeleton. The usual pattern for this genus is the polymethoxylation of the flavonoids, but in this species we identified only two trimethoxylated derivatives, while we found three flavonoid *C*-glycosides which, instead, are usually quite rare in the genus *Centaurea*, having been isolated only in few species so far [6,9,10].

Fractionation of R_C residue led to the purification of compound **1**, the new identified *nor*-guaianolide. It was obtained as a pale yellowish powder that appears on TLC as a brownish spot after treatment at 120 °C with cerium sulfate spray reagent. The positive ESI-MS spectrum gave a *quasi-molecular* peak $[M - H]^+$ at 251 *m*/*z*, corresponding to the molecular formula $C_{14}H_{18}O_4$, supported also by elemental analysis. The ^{13}C NMR spectrum showed 14 signals, sorted by DEPT experiments into 6 CH, 4 CH_2, 1 CH_3 and 3 quaternary C. The ^1H NMR spectrum of **1** suggested a non-α-exo-methylene sesquiterpene lactone structure in C11–C13. This situation was clearly visible because of the lack of the two typical doublets ($J \approx 3$ Hz) between 5.5 and 6.5 ppm. Typical signals observable in the ^1H NMR spectrum were the two one-proton broad singlets at 5.09 and 4.80 ppm attributable to the vinyl hydrogens linked to the sp^2 hybridized C-14. This exocyclic double bond was supported by the signals at 116.3 and 143.5 ppm in the ^{13}C NMR spectrum (sorted as a methylene and a quaternary carbon by DEPT experiments, respectively). Two further deshielded signals (a multiplet at 4.19 ppm and a broad double doublet at 4.22) were present in the ^1H NMR spectrum, indicating the proximity of a heteroatom, likely an oxygen. By mean of 2D-NMR experiments they were attributed to H-8 and H-6, respectively (Fig. 1a and b). In the NOESY spectrum a correlation between the signal at 3.11 ppm (H-1) and that at 2.28 ppm (H-5) was observed, indicating the *cis* junction of the two rings. On the contrary, no correlation was observed between the signals at 2.28 and 4.22 ppm (H-5 and H-6, respectively), suggesting a *trans* relationship of these protons. This also permitted to establish an α substitution for the oxygen of the lactone ring. The high coupling constant (9.8 Hz) between H-6 and H-7 (4.22 and 2.24 ppm, respectively) suggest another *trans* relationship of these protons, clarifying the stereochemistry of C-7. The NOE effect between the ddd at 2.24 ppm (H-7) and the doublet at 1.22 ppm (13-methyl group) permitted to determine the α substitution for the methyl group. Finally, the NOE effect between the signals at 2.31 and 4.19 ppm (H-11 and H-8) clarified the α substitution for the OH group linked to C-8. Further signals observed in the ^{13}C NMR were the ketone at 219.0 ppm, the lactone at 175.9 ppm and the two carbinolic methines at 70.5 and 82.8 ppm. Furthermore, only 14 signals were present instead of the 15 expected. Excluded the signal overlapping, also according to mass and elemental analysis, a *nor*-sesquiterpene has been hypothesized, and more precisely a *nor*-guaianolide one. On the basis of 2D-NMR data (COSY, HMQC, HMBC) the structure has been elucidated as **1** (Fig. 1a and b).

From a literature survey, sesquiterpene lactones result in the main class of substances isolated from the genus *Centaurea*, but among them, *nor*-derivatives are very rare in nature as demonstrated by the isolation of only four derivatives from other Asteraceae genera of which only two were 15-*nor*-guaianolides [11–13]. Probably they could derive from an oxidative loss of the C-15 methyl group of the parent guaianolide (i.e. isoamberboin), as observed in related skeletons such as those of guaianes [14]. A similar behavior is also reported for other chemical classes of secondary metabolites, such as iridoids, by oxidative decarboxylation of methyl groups on the skeleton [15].

Sesquiterpene lactones have a well-known recognised potential as anticancer agents, indeed three of them are currently under clinical investigation [16]. Further molecules from this class may represent agents to be used as new lead compounds or to give insights about the chemical substituents relevant for the anticancer activity. So, the biological activity of *C. deflexa* was investigated through a bio-guided assay fractionation measuring the anti-proliferative effect of plant extracts and fractions on two human cancer cell lines, the highly chemo-resistant pancreatic MIA PaCa-2 and the moderate chemo-resistant colonic COLO 320. The results showed a consistent time- and concentration-dependent effect on both cell lines after treatment with lipophilic extracts revealing also a correlation trend between the anti-proliferative activity of plant extracts and their lipophilicity (Fig. E2 Supplementary Information). Consequently, the further evaluation of biological activity of *C. deflexa* was mainly focused on compounds isolated from the more lipophilic extracts and fractions. Amongst all the compounds assessed, two sesquiterpene lactones, aguerin B and the new identified 15-*nor*-guaianolide (**1**), exhibited the highest anti-proliferative effect on both human cancer cell lines in a concentration- and time-dependent manner. Aguerin B (Fig. 2) showed a good anti-proliferative effect as demonstrated by IC_{50} values significantly lower than those obtained with 5-fluorouracil (5-FU), a drug commonly used in colorectal and pancreatic

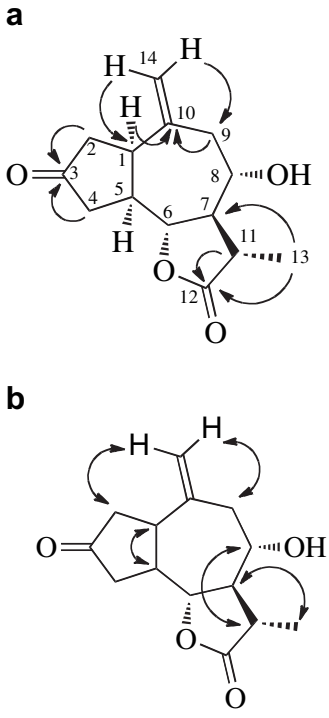

Fig. 1. (a) 15-*nor*-guaianolide (**1**), significant HMBC correlations; (b) 15-*nor*-guaianolide (**1**), significant NOESY correlations.

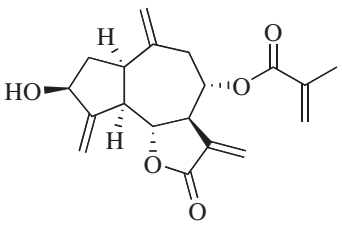

Fig. 2. Chemical structure of aguerin B.