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Phloroglucinol derivatives from *Hypericum empetrifolium* with antiproliferative activity on endothelial cells

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ABSTRACT

Five acylphloroglucinols substituted with monoterpenoids (empetrifelixin A–D and empetrikajaforin), three known monocyclic acylphloroglucinols and one monocyclic acylphloroglucinol were isolated from a petrol ether extract of *Hypericum empetrifolium* after fractionation by flash chromatography on silica gel, RP-18 and subsequent purification by preparative HPLC (RP-18). Their structures were elucidated by 1D, 2D NMR techniques and HREIMS. To determine a possible anti-angiogenic activity, inhibition of cell proliferation was measured using a human microvascular endothelial cell line (HMEC-1). Subconfluent grown HMEC-1 cells were treated with all compounds isolated in sufficient amounts and stained with crystal violet. Highest activity was observed for empetrifelixin A and empetrifelixin D showing a concentration dependent inhibition of cell proliferation with IC_{50} values of 6.5 ± 0.1 and 7.3 ± 0.4 μM, respectively. Empetrifelixin A also showed activity in a cell migration assay with HMEC-1 cells in low micromolar concentrations.

1. Introduction

Acylphloroglucinols are typical secondary metabolites accumulated in the families Hypericaceae (Avato, 2005) and Clusiaceae. The phloroglucinol core of these derivatives is often substituted by several prenyl or geranyl moieties. Both were able to undergo cyclization and oxidation processes resulting in bi- (Henry et al., 2006; Winkelmann et al., 2001) or tricyclic (Chen et al., 2010; Winkelmann et al., 2000) as well as complex caged compounds (Hu and Sim, 1999; Ishida et al., 2010). Among both families the genera *Garcinia*, *Clusia* and *Vismia* can be characterized by the dominance of phloroglucinols with an aromatic acyl moiety, whereas the genus *Hypericum* mainly (with several exceptions) accumulates derivatives exhibiting aliphatic acyl moieties. A further interesting, but rare, structural variation is the occurrence of phloroglucinols with monoterpenoid substitution (An et al., 2002; Hashida et al., 2008).

The structural diversity of phloroglucinols resulted in various pharmacological activities *in vitro* and *in vivo*. Several representatives have been reported to exert significant antibacterial activities especially against gram-positive bacteria (Rocha et al., 1995; Shiu and Gibbons, 2006). Also, a moderate to strong cytotoxicity against different tumor cell lines has been observed (Hashida et al., 2008; Hu and Sim, 1999; Momekov et al., 2008). Further derivatives have been proven to exhibit anti-oxidative effects (Heilmann et al., 2003). Hyperforin, the main acylphloroglucinol of St. John's wort (*Hypericum perforatum* L.), has been reported to show inhibition of key enzymes of the arachidonic acid cascade (Albert et al., 2002) or transcription factors (Kraus et al., 2010). Moreover, it is supposed to contribute to the antidepressant efficacy of St. John's wort extracts (Butterweck, 2003). Recently, a potent anti-angiogenic activity of hyperforin has been described (Schempp et al., 2005) and derivatives with higher stability have been semi-synthesized and tested (Martínez-Poveda et al., 2010).

The aim of the present study was the identification of acylphloroglucinols from *Hypericum empetrifolium* (Hypericaceae) and to evaluate their anti-proliferative *in vitro* activity on an endothelial cell line. This *in vitro* activity is likely a first hint for characterization of an anti-angiogenic activity. The most active compound was also tested due to its ability to reduce cell migration in a migration (scratch) assay.

H. empetrifolium is primarily found in Turkey and Greece and used in the traditional medicine of both countries. Tuzlacy (2006) reported on the treatment of kidney stones and gastric ulcers in Turkey, whereas Vokou et al. (1993) described the external use as a wash to speed wound-healing, heal scalds, and treat outbreaks of herpes. An extract of *H. empetrifolium* has been reported to exert analgetic and anti-inflammatory activity (Trovato et al., 2001). Consequently, Crockett et al. (2008) isolated two acylphloroglucinols (**7**, **8**) with moderate to potent *in vitro* activity against COX-1, COX-2 or 5-LOX. A following phytochemical investigation on the title plant focused on the presence of hypericin derivatives (Alali et al., 2009).

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As several members of the acylphloroglucinol family show a free hydroxyl in the vicinity of the acyl moiety our approach for the systematic identification and isolation of these compounds was guided by ^{1}H NMR. The protons of the hydroxyl groups, which are involved into a strong hydrogen bond with the acyl substituent are characteristically downfield shifted to $\delta_H \geqslant 11$ ppm and thus easily detectable in a fraction.

2. Results and discussion

All compounds **1**–**9** (Fig. 1) were isolated by detection of their downfield shifted proton signals ($\delta_H > 11$ ppm) except compound **6** (δ_H 9.72 ppm) in ^{1}H NMR spectrum and isolated as viscous oils. The HREIMS spectrum of **1** revealed a $[M]^+$ at *m/z* 468.3237 pointing to a molecular formula of $C_{30}H_{44}O_4$ (calcd. 468.3240). The ^{13}C NMR spectrum showed 30 carbon signals, which could be sorted by HSQC into eight methyl, six methylene, six methine, and 10 quaternary carbons. The presence of a phloroglucinol skeleton was deduced from the carbon shift values of C-1–C-6 (Table 1). Among these signals, three downfield shifted carbons δ_C 163.9 ppm (C-3), 160.6 ppm (C-5), 157.6 ppm (C-1) substantiated the trihydroxylation. Two quaternary carbons (δ_C 107.7 ppm C-2; 106.3 ppm C-4) pointed to substitution with aliphatic side chains and one signified a methine at C-6 (δ_C 98.0 ppm; δ_H 6.06 ppm, s).

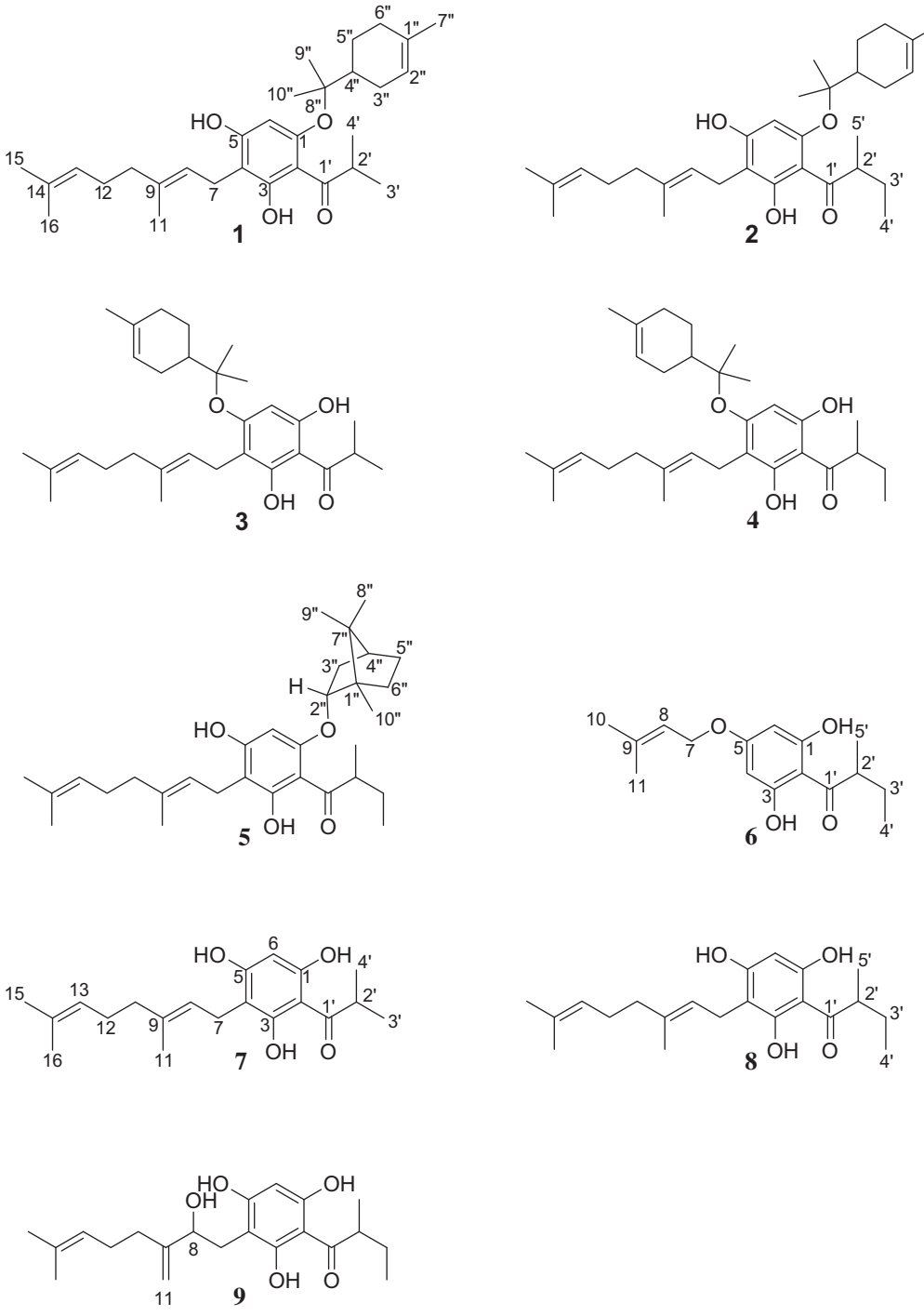

Fig. 1. Structures of compounds **1**–**9**.