NPC Natural Product Communications

2006
Vol. 1
No. 1
33 - 35

# Hederacine A and Hederacine B from *Glechoma hederaceae* Inhibit the Growth of Colorectal Cancer Cells *in vitro*

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**Received: October 10th, 2005; Accepted: November 3rd, 2005**

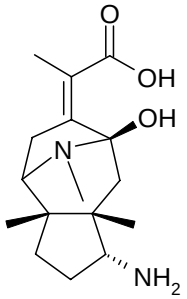
The cytotoxic activity of the tropane alkaloids, hederacine A (**1**) and hederacine B (**2**), previously isolated from the methanol extract of the aerial parts of *Glechoma hederaceae*, was assessed by the MTT cytotoxicity assay using colon cancer cell line (CaCo-2). The $IC_{50}$ values for **1** and **2** were found to be 86.6 and 301.0 μM, respectively.

**Keywords:** *Glechoma hederaceae*, Lamiaceae, hederacine A, hederacine B, tropane alkaloid, colon cancer, MTT.

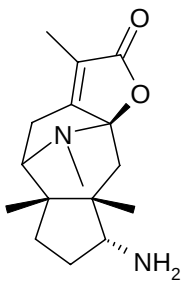
In 2000, there were 10 million new cases, 7 million deaths and 22 million people living with various forms of cancers, the most common being lung, breast, colorectal, stomach and prostate/uterus cancers. Colorectal cancer is a fatal malignancy, being second to lung cancer as a cause of cancer-death in Scotland. Currently available treatment includes surgical resection as the primary curative modality, with endoscopic resection representing another approach for the small minority of early malignant polyp-cancers [1]. As part of our on-going search for novel 'leads' from higher plants for the development of new drugs to treat colon cancers [2], we now report on the cytotoxic properties of two tropane alkaloids, hederacine A (**1**) and hederacine B (**2**), isolated from the aerial parts of *Glechoma hederaceae* [3], towards the colon cancer cell line (CaCo-2) using MTT cytotoxicity assay [4].

*Glechoma hederacea* L. (Lamiaceae alt. Labiatae), commonly known as 'ground ivy', 'creeping Charlie', 'runaway Robin' or 'gill-over-the-ground' is a perennial hairy herb with creeping stem, from a few inches to 1–2 ft long [3]. This plant is common to temperate Asia, Europe and the USA, where it is found growing in shady places, waste grounds, dry ditches, fences and hedges, and on the sides of moist meadows. Previous phytochemical investigations on this plant revealed the presence of a variety of secondary metabolites including alkaloids, flavonoids, phenyl propanoids, saponins and terpenoids [3].

Hederacine A (**1**) and hederacine B (**2**) were isolated from the methanol extract of the aerial parts of *G. hederaceae* by a combination of solid-phase extraction and reversed-phase preparative HPLC [3].




**1**




**2**

The structures of these compounds were determined by extensive spectroscopic analyses [3]. In the brine shrimp lethality assay [5] these compounds were reported to display significant general toxicity, similar to that of the known cytotoxic lignan, podophyllotoxin [3], and it was assumed that these compounds might be cytotoxic towards cancer cells. The present study on the assessment of cytotoxic properties of these compounds (**1** and **2**) in colon cancer cell line, CaCo-2, has proved this preliminary assumption. The *in vitro* cytotoxicities of **1** and **2** were determined by the MTT cytotoxicity assay [4], and the $IC_{50}$ values were found to be 86.6 and 301.0 μM, respectively (Table 1). The main structural difference between **1** and **2** is the presence or absence of the cyclic ester moiety (as in **2**). The presence of a free hydroxyl and a free carboxylic acid functionalities in **1**, as opposed to the cyclic ester in **2**, might have contributed to the enhanced (>3 times) cytotoxicity of hederacine A (**1**) compared to that of **2**. As these alkaloids (**1** and **2**) showed moderate levels of anti-cancer activity, these compounds, in their present form, might not be suitable candidates for the development of therapeutically significant and commercially viable anti-cancer drugs. However, their unique structural features can certainly be utilized as a template for generating compounds with enhanced activity. While this is the first report on the anti-colon cancer activity of any tropane alkaloids, several tropane alkaloid esters, isolated from the roots of *Erythroxylum pervillei,* showed potential to reverse multidrug-resistance with vinblastine-resistant oral epidermoid carcinoma (KB-V1) cells [6, 7].

**Table 1**: Cytotoxicity of hederacine A (**1**) and hederacine B (**2**), isolated from *G. hederaceae*, against colon cancer cell-line (CaCo-2)

| Compounds | Cytotoxicity $IC_{50}$ (μM) |
|---|---|
| **1** | 86.8 |
| **2** | 301.0 |
| Podophyllotoxin | 0.06 |

## Experimental

***General:*** UV spectra were obtained using a Hewlett–Packard 8453 UV–Vis spectrometer. An AVATAR 360 FT-IR spectrometer was used for obtaining IR spectra. NMR spectra were recorded in $CD_3OD$ on a Varian Unity INOVA 400 MHz NMR Spectrometer 400 (400 MHz for $^1H$ and 100 MHz for $^{13}C$) using the residual solvent peaks as internal standard. HRMS on a Quattro II triple quadrupole instrument HPLC separation was performed in a Dionex prep-HPLC System coupled with Gynkotek GINA50 autosampler and Dionex UVD340S Photo-Diode-Array detector. A Luna C18 preparative HPLC column (10 μ x 250 mm x 21.2 mm) was used. Sep-Pak DSC-18 Supelco 10 g cartridge was used for pre-HPLC fractionation. HMBC spectra were optimized for a long-range $J_{H-C}$ of 9 Hz and NOESY experiment was carried out with a mixing time of 0.8 s.

***Plant material:*** Seeds of this plant (cat. no. 15105) were purchased from B & T World Seeds sarl, Paguigan, 334210 Olonzac, France. Plants were grown from these seeds in the greenhouse, harvested during March 2002 and a voucher specimen (PH100010) has been deposited in the herbarium of Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

***Extraction and isolation:*** The dried, ground plant material (135 g) was Soxhlet-extracted, successively, with *n*-hexane, dichloromethane (DCM) and methanol (MeOH). The isolation protocols for these compounds were described elsewhere [3].

**Hederacine A** (**1**)

Yellow amorphous solid.
4.6 mg (yield 0.0034%).
IR $\nu_{max}$ (NaCl): 3356, 2924, 2360, 1715, 1515, 1455, 1161, 1071 $cm^{-1}$.
UV $\lambda_{max}$ (MeOH) nm (log ε): 232 (4.00).
$^1H$ and $^{13}C$ NMR: [3].
HR-MS: $C_{16}H_{30}N_3O_3$ $[M+NH_4]^+$ requires 312.22869 (found 312.22860).

**Hederacine B** (**2**)

Yellow amorphous solid.
3.1 mg; yield 0.00229%.
IR $\nu_{max}$ (NaCl): 34426, 2922, 2850, 1765, 1512, 1455, 1159, 1062 $cm^{-1}$.
UV $\lambda_{max}$ (MeOH) nm (log ε): 233 (3.98).
$^1H$ and $^{13}C$ NMR: [3].
HR-MS: $C_{16}H_{28}N_3O_2$ $[M+NH_4]^+$ requires 294.21813 (found 294.21811).

***MTT cytotoxicity assay:*** CaCo-2 cells were maintained in Earle's minimum essential medium (Sigma), supplemented with 10% (v/v) fetal calf serum (Biosera), 2 mM L-glutamine (Sigma), 1% (v/v) non-essential amino acids (Sigma), 100 IU/mL penicillin and 100 mg/mL streptomycin (Sigma). Exponentially growing cells were seeded with 10,000

cells per well into a 96-well plate (Nunc) and incubated for 72 h before the addition of drugs. Stock solutions of compounds were initially in DMSO or $H_2O$ and further diluted (0.5 μM to 400 μM) with fresh complete medium. The growth-inhibitory effects of the compounds (1 and 2) were measured using standard tetrazolium MTT assay [4]. After 72 h of incubation at 37°C, the medium was removed and 100 μL of MTT reagent (1 mg/mL) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 h. At the end of the incubation period, the medium was removed and pure DMSO (200 μL) was added to each well. The metabolized MTT product dissolved in DMSO was quantified by reading the absorbance at 560 nm on a microplate reader (Dynex Technologies, USA). The assay was performed in triplicate. The $IC_{50}$ values were calculated from the equation of the logarithmic line determined by fitting the best line (Microsoft Excel) to the curve formed from the data.

**Acknowledgment** - We thank the EPSRC National Mass Spectrometry Service Centre (Department of Chemistry, University of Wales Swansea, Swansea, Wales, UK) for MS analyses.

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