

## SHORT COMMUNICATION

# NF- $\kappa$ B Modulators from *Valeriana officinalis*

Nadia J. Jacobo-Herrera<sup>1†</sup>, Nina Vartiainen<sup>2</sup>, Paul Bremner<sup>1‡</sup>, Simon Gibbons<sup>1</sup>, Jari Koistinaho<sup>2,3</sup> and Michael Heinrich<sup>1\*</sup>

<sup>1</sup>Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, UK

<sup>2</sup>Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Finland

<sup>3</sup>Department of Oncology, Kuopio University Hospital, Kuopio, Finland

*Valeriana officinalis* (Valerianaceae) has been of great interest for its therapeutic uses for treating mild nervous tension and temporary sleeping problems. In traditional European medicine it has been also reported as an antiinflammatory remedy. This study reports that the EtOAc extract of the underground parts of *V. officinalis* showed inhibitory activity against NF- $\kappa$ B at 100  $\mu$ g/mL in the IL-6/Luc assay on HeLa cells and provided protection against excitotoxicity in primary brain cell cultures at micromolar concentrations. Bioassay-guided fractionation of the EtOAc extract led to the isolation of three known sesquiterpenes: acetylvalerenolic acid (**1**), valerenal (**2**) and valerenic acid (**3**), **1** and **3** were active as inhibitors of NF- $\kappa$ B at a concentration of 100  $\mu$ g/mL. Acetylvalerenolic acid (**1**) reduced NF- $\kappa$ B activity to 4%, whereas valerenic acid (**3**) reduced NF- $\kappa$ B activity to 25%. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: NF- $\kappa$ B; valerian; traditional (European) medicine; sesquiterpenes; HeLa cells; neurons.

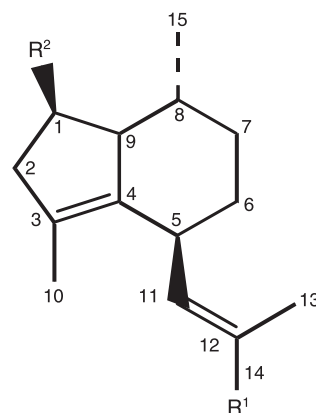
## INTRODUCTION

*Valeriana officinalis* L. (Valerianaceae), commonly known as valerian, is probably one of the most popular medicinal herbs used throughout human history. The reputation of this plant in modern rational phytotherapy is mainly due to its pharmacological properties as a mild sedative, and for treating mild nervous tension and temporary sleeping problems. The active compounds for these effects, however, remain a matter of great controversy (Bisset and Wichtl, 2001). The interest in studying *V. officinalis* as an inhibitor of NF- $\kappa$ B emerged from the knowledge of its traditional use as an antiinflammatory remedy in Europe and the use of related species in Central America/Mexico for similar purposes (Cáceres, 1999; Mayer, 2003). Therefore, the aim of the present study was to investigate the effects of an EtOAc extract of *V. officinalis* on neurophysiological pathologies linked with the NF- $\kappa$ B pathway (Bremner and Heinrich, 2005) and through bioassay-guided fractionation to isolate fractions and compounds from an active EtOAc extract of *V. officinalis* using the IL-6/luciferase assay as a lead. In particular, the study focused on the potential protective effect of the EtOAc extract against excitotoxicity, which is known

to contribute to the pathogenesis of stroke and neurodegenerative diseases.

## MATERIAL AND METHODS

**Plant material.** Rootstock of *V. officinalis* was commercially supplied by Potter's Herbal Medicines® (Leyland Mill Lane Wigan, Lancs WN1 2SB, UK). Powdered plant material (400 g) was extracted by cold maceration with ethyl acetate (2 L) at room temperature. The solvent was removed under vacuum and dried with a rotavapor to give a total crude extract of 9 g which was stored at  $-20^{\circ}\text{C}$ . Compound **1** (6 mg), acetylvalerenolic acid (acetoxyvalerenic acid), was isolated by two different chromatographic techniques: (a) vacuum liquid chromatography on silica gel column



	R <sup>1</sup>	R <sup>1</sup>	
<b>1</b>	COOH	OAc	Acetylvalerenolic acid
<b>2</b>	CHO	H	Valerenal
<b>3</b>	COOH	H	Valerenic acid

\* Correspondence to: Professor Michael Heinrich, Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK.

E-mail: michael.heinrich@pharmacy.ac.uk

† Current address: Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico.

‡ Current address: Faculty of Health and Life Sciences, De Montford University, Leicester, UK.

Contract/grant sponsor: Consejo Nacional de Ciencia y Tecnología (CONACyT, México).

Contract/grant sponsor: Secretaría de Educación Pública (SEP, México).

Contract/grant sponsor: European Union; contract/grant number: Framework 5, QLK3-2000-00 463, AINP.

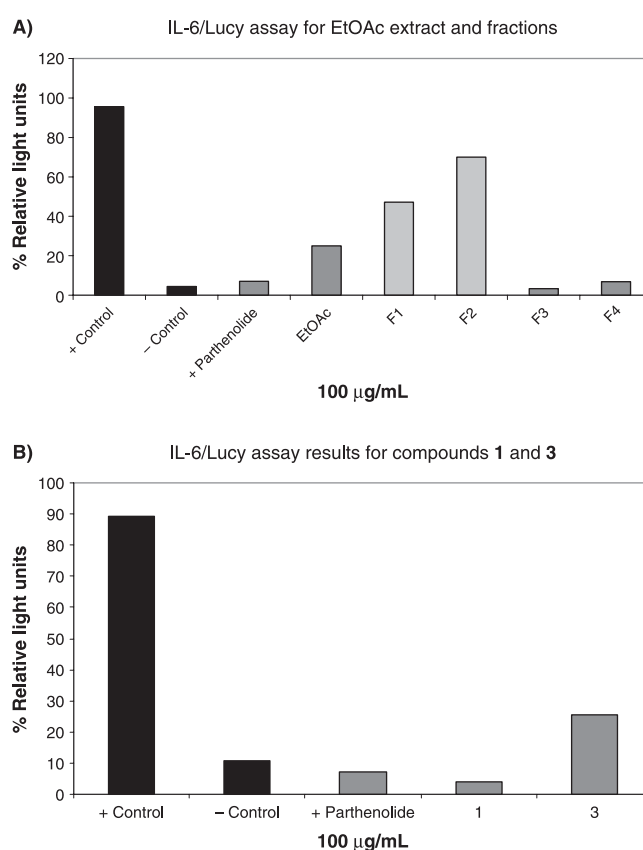
Received 2 June 2006  
Accepted 16 June 2006

(5 g of crude extract) using a hexane–EtOAc gradient elution system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10; 100 mL each), and purified by preparative TLC [hexane–EtOAc 7:3, 1:2;  $R_f = 0.59$  (hexane–EtOAc 1:2)]; and (b) preparative HPLC (gradient, 100% H<sub>2</sub>O–100% ACN; retention time 31 min). Compound **2** (9 mg), valerenol, was isolated by a solid phase extraction gradient system, using hexane–CHCl<sub>3</sub> as eluent (increments of 5% starting 10:0) and preparative TLC [hexane–CHCl<sub>3</sub> 3:2;  $R_f = 0.65$  (hexane–CHCl<sub>3</sub> 1:1)]. Compound **3** (6 mg), valeric acid, was isolated by Sephadex LH-20 (4 g of crude extract, EtOH as mobile phase) and preparative TLC [hexane–EtOAc 7:3;  $R_f = 0.52$  (hexane–EtOAc 1:2)]. Characterization of compounds was achieved using <sup>1</sup>H and <sup>13</sup>C NMR spectra in CDCl<sub>3</sub> and comparing the data obtained with those from previously published spectral data (Bos *et al.*, 1986; Dharmaratne *et al.*, 2002). Copies of the original spectra can be obtained from the corresponding author upon request.

**Bioassay.** To test the effect of the extract on neuronal injury, primary cortical cultures with and without glia were prepared as described by Koponen *et al.* (2003) and then exposed to various concentrations of NMDA and kainate for 24 h before measuring the neuronal death using an LDH assay with the Sigma kinetic LDH kit. The EtOAc extract dissolved in 0.05% DMSO was added to the cultures 30 min before the exposure. To test for the activity of fractions and compounds as inhibitors of NF- $\kappa$ B, a luciferase assay was employed using HeLa cells. Briefly, the cells were stably transfected with a luciferase reporter gene controlled by the IL-6 promoter. The stimulant phorbol 12-myristate-13-acetate (PMA) was added to a final concentration of 50 ng/mL and the plates were incubated for 7 h. The luciferase value had to be at least 30% of the positive control to be considered active. Readings were taken with an Anthos Lucy 1 luminometer/photometer. Toxicity was evaluated visually under magnification (40 $\times$ ) by morphological changes in the cells. The full methodology followed in this work for the IL-6/luciferase assay is described in detail elsewhere (Bremner *et al.*, 2004).

## RESULTS AND DISCUSSION

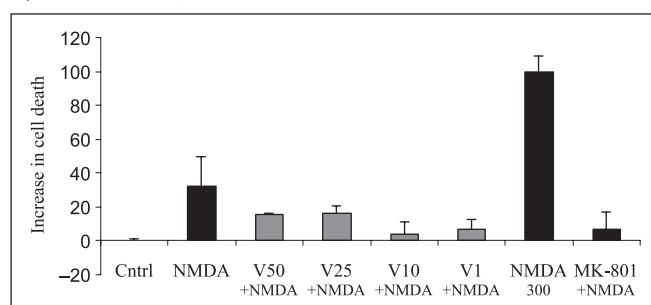
The EtOAc extract obtained from the rootstock of *V. officinalis* inhibited NF- $\kappa$ B activation to 25% at



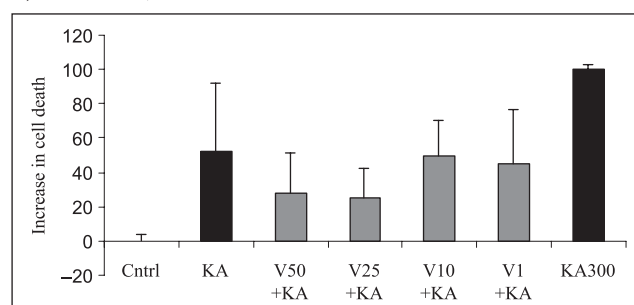
**Figure 1.** Inhibitory effect on NF- $\kappa$ B activation of *V. officinalis* induced by PMA in HeLa cells stably transfected with a luciferase reporter gene controlled by the IL-6 promoter. Treatment values are given as a percentage of relative light units compared with the stimulated control (positive) (mean data of three experiments). Positive control consisted of stimulated cells with PMA. Negative control contains resting cells without stimulation. Parthenolide was used as a positive control as inhibitor of NF- $\kappa$ B at 2.5 µg/mL. (A) Inhibitory activity against NF- $\kappa$ B of EtOAc extract and fractions (F1–F4) at 100 µg/mL. (B) Inhibitory activity against NF- $\kappa$ B of compounds acetylvalerenolic acid (**1**) and valeric acid (**2**) at 100 µg/mL.

100 µg/mL (Fig. 1A). In primary neuronal cultures, that contained microglia and astrocytes in addition to neurons, measured as release of lactate dehydrogenase 24 h after exposure, the EtOAc extract reduced the *N*-methyl-D-aspartate (NMDA) and kainate-induced excitotoxicity at 1–50 µM and 25–50 µM concentration, respectively (Fig. 2). In pure neuronal cultures the EtOAc extract did not protect the neurons against either toxin; instead, the extract alone increased neuronal death at

**A)** NMDA 35 mM, 5 min + 24 h



**B)** KA 50 mM, 24 h



**Figure 2.** Mixed cortical cultures: significant neuroprotection with valerian extract against: (A) NMDA toxicity at concentrations of 1, 10, 25 and 50 µM (V1, V10, V25 and V50, respectively), and (B) kainate at concentrations of 25 and 50 µM (V25 and V50, respectively). Extracts were added 30 min prior to excitotoxins. The neuronal cell death was determined after 24 h.

50  $\mu$ M concentration. These results indicate that the neuroprotective effect of the EtOAc extract is dependent on the presence of glial cells, which are known to become activated upon brain injury and neurodegenerative disease and to promote harmful inflammatory reactions (Tikka and Koistinaho, 2001; Koistinaho and Koistinaho, 2005), an effect in part due to NF- $\kappa$ B activation.

The EtOAc extract was fractionated into four large fractions by VLC. The four fractions were tested at 100  $\mu$ g/mL using the IL-6/luciferase assay. Fractions 3 and 4 reduced NF- $\kappa$ B activity to 3% and 7%, respectively (Fig. 1A); although fraction 3 was 50% cytotoxic to HeLa cells after 7 h of exposure. The toxicity was observed under a microscope by detecting morphological changes in cells and/or cell death. Compound **1** reduced NF- $\kappa$ B activity to 4% at 100  $\mu$ g/mL (342.03  $\mu$ M) (Fig. 1B). Compound **3** was shown to reduce NF- $\kappa$ B activity to 25% at 100  $\mu$ g/mL (426.74  $\mu$ M) with no visible cytotoxic effects (Fig. 1B). Compound **2** did not show any inhibitory activity at the same concentration.

Fractions where compounds **1** and **3** were present had higher inhibitory activity than the two compounds alone (Fig. 1). This result may suggest that there could be a synergistic or additive effect with other constituents in the fraction that increase the inhibitory activity of the fractions. It has also been proposed that the tranquillizer and mild sedative properties of *V. officinalis* rely on the combined sesquiterpenes present in the volatile oil, which are mainly a combination of valerenic acid (**3**), acetoxylvalerenic acid and hydroxyvalerenic acid (Boyadzhiev *et al.*, 2004). Other studies have shown that the sesquiterpene valerenic acid has spasmolytic and muscle relaxants properties (Birnbaum *et al.*, 1978;

Bisset and Wichtl, 2001), while valeranone exhibits tranquillizing effect (Birnbaum *et al.*, 1978). Some studies on the other hand, have revealed that the valepotriates possess tranquillizing effects in mice and cats, but also have shown to be mutagenic and carcinogenic *in vitro* due to the presence of an epoxide group and its alkylating potential (Bounthanh *et al.*, 1981; Braun *et al.*, 1982). The latter may be linked to the inhibitory effects observed in this study, since cytotoxic effects were observed in some fractions and subfractions but not in pure compounds. With regard to valepotriates, it has been shown that valtrate, dihydrovaltrate and deoxido-didrovaltrate have a cytotoxic effect on cell growth and viability on rat hepatic cells (HTC strain), with valtrate being the most active (Bounthanh *et al.*, 1983). Due to the chemical complexity of fractions, it is possible that other compounds, such as valepotriates, were responsible for this cytotoxicity. The present study provides biochemical evidence for the use of *V. officinalis* as an antiinflammatory agent acting via the NF- $\kappa$ B inhibitory potential of compounds **1** and **3**. Further studies assessing the pro-apoptotic/cytotoxic effects of the crude extracts, fractions and compounds obtained from *V. officinalis* should be performed in order to clarify the NF- $\kappa$ B inhibitory mechanism of action.

#### Acknowledgement

We thank to Consejo Nacional de Ciencia y Tecnología (CONACyT, México) and Secretaría de Educación Pública (SEP, México) for funding the PhD and providing a supplementary studentship, respectively, to NJJ-H. Funding from the European Union (Framework 5, QLK3-2000-00 463, AINP) is gratefully acknowledged. We thank Dr Rogelio Pereda-Miranda for useful discussions and comments on the text.

#### REFERENCES

- Birnbaum GI, Findlay JA, Krepinsky JJ. 1978. Stereochemistry of valerenane sesquiterpenoids, crystal structure of valerenolic acid. *J Org Chem* **43**: 272–276.
- Bisset NG, Wichtl M. 2001. *Herbal Drugs and Phytopharmaceuticals. A Handbook for Practice on a Scientific Basis with Reference to German Commission E Monographs*, 2nd edn. Medpharm Scientific Publishers: Germany, 513–519.
- Bos R, Hendriks H, Bruins AP, Kloosterman J, Sipma G. 1986. Isolation and identification of valerenane sesquiterpenoids from *Valeriana officinalis*. *Phytochemistry* **25**: 133–135.
- Bounthanh C, Bergmann C, Beck JP, Haag-Berrurier M, Anton R. 1981. Valepotriates, a new class of cytotoxicity and antitumor agents. *Planta Med* **41**: 21–28.
- Bounthanh C, Richert L, Beck JP, Haag-Berrurier M, Anton R. 1983. The action of valepotriates on the synthesis of DNA and proteins of cultured hepatoma cells. *Planta Med* **49**: 138–142.
- Boyadzhiev L, Kancheva D, Gourdon C, Metcheva D. 2004. Extraction of valerenic acids from valerian (*Valeriana officinalis* L.) rhizomes. *Pharmazie* **59**: 727–728.
- Braun R, Dittmar W, Machut M, Weickmann S. 1982. Valepotriate mit Epoxidstruktur-beachtliche Alkylantein. *Dtsch Apoth Ztg* **122**: 1109–1113.
- Bremner P, Heinrich M. 2005. Natural products and their role as inhibitors of the pro-inflammatory transcription factor NF- $\kappa$ B. *Phytochem Rev* **4**: 27–37.
- Bremner P, Tang S, Birkmayer H *et al.* 2004. Phenylpropanoid NF- $\kappa$ B inhibitors from *Bupleurum fruticosum*. *Planta Med* **70**: 1–5.
- Cáceres A. 1999. *Plantas de Uso Medicinal en Guatemala*. Editorial Universitaria: Guatemala.
- Dharmaratne HRW, Nanayakkara NPD, Khan IA. 2002. (-)-3 $\beta$ , 4 $\beta$ , Epoxyvalerenic acid from *Valeriana officinalis*. *Planta Med* **68**: 661–662.
- Koistinaho M, Koistinaho J. 2005. Interactions between Alzheimer's disease and cerebral ischemia – focus on inflammation. *Brain Res Rev* **48**: 240–250.
- Koponen S, Kurkinen K, Akerman KE, Mochly-Rosen D, Chan PH, Koistinaho J. 2003. Prevention of NMDA-induced death of cortical neurons by inhibition of protein kinase. *Czet J Neurochem* **86**: 442–450.
- Mayer J. 2003. Zur Geschichte des Baldrian und Hopfen. Eine moderne Verbindung alter Arzneipflanzen? *Z Phytother* **2**: 70–82.
- Tikka TM, Koistinaho J. 2001. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* **166**: 7527–7533.