Suzanne Lim[1, 2]
Jérôme Grassi[3]
Valentina Akhmedjanova[4]
Eric Debiton[1, 2]
Guy Balansard[5]
Richard Beliveau[3]
Chantal Barthomeuf[1, 2]

Reversal of P-Glycoprotein-Mediated Drug Efflux by Eudesmin from *Haplophyllum perforatum* and Cytotoxicity Pattern versus Diphyllin, Podophyllotoxin and Etoposide

Abstract

The present study focuses on eudesmin (bicyclic lignan, 0.15 % of dry leaves) and diphyllin (arylnaphthalene lignan, 0.1 % of dry roots), both isolated from *H. perforatum* Kar. et Kir, a Rutaceae species endemic to Uzbekistan. We first compared their specificity for cancer cells with those of etoposide and podophyllotoxin by screening their cytotoxicity on 3 healthy cell-lines and 7 sensitive or resistant human solid cancer lines. We then tested their capacity to reverse P-glycoprotein-mediated multidrug resistance (MDR) by assaying dye and drug uptake in MDR1-transfected Madin-Darby canine kidney (MDCK-MDR1) and doxorubicine-resistant human breast carcinoma cells (MCF7/Dox). Eudesmin displays IC_{50} values > 100 M on all tested lines. Our data provide the first demonstration that this non-toxic lignan reverses Pgp-mediated drug efflux and supports the hypothesis that it may inhibit resistance mediated by MDR1 and MRP proteins. Even if its reversal activity is insufficient for clinical application, its capacity to accumulate [^3H]-vinblastine in MDCK/MDR1 and MCF7/Dox cells suggests that eudesmin may positively affect the bioavailability and, thereby, the therapeutic potency of anticancer drugs in Pgp-overexpressing cells. Diphyllin exhibits IC_{50} values ranging from 10^{-6} to 10^{-4} M. It is markedly less toxic than podophyllotoxin (IC_{50} : 13 – 61 nM), but exhibits tumoricidal effects close to those of etoposide. Unfortunatly, it is 65-fold more toxic than etoposide on human primary fibroblasts. Consequently, it has no value as an anticancer drug. Its value as raw material for the hemisynthesis of anticancer drugs is discussed.

Key words
Haplophyllum perforatum · Rutaceae · lignans · diphyllin · eudesmin · P-glycoprotein · cytotoxicity

Introduction

Prior to the discovery of the microtubule inhibitory activity of podophyllotoxin, lignans and in particular arylnaphthalene lignans have attracted strong attention from researchers working in the field of oncology. Species from the genus *Haplophyllum* (Rutaceae) used primarily in folk medicine in Central Asia to treat warts, tumours of testes, lichens, herpes, erysipelas and some painful syndromes from different origins are rich in alkaloids and lignans [1], [2], [3], [4]. This study focuses on eudesmin

Affiliation
[1] INSERM-484, Clermont-Ferrand, France
[2] Laboratoire de Pharmacognosie et Biotechnologies, Faculté de Pharmacie, Université d'Auvergne, Clermont-Ferrand, France
[3] Laboratoire de Médecine Moléculaire, Hôpital Ste-Justine-UQAM, Montréal, Québec, Canada
[4] Laboratory of Alkaloids Chemistry, Yunusov Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan
[5] Laboratoire de Pharmacognosie et Phytothérapie, Faculté de Pharmacie, Université Aix-Marseille II, Marseille, France

Correspondence
Prof Chantal Barthomeuf · INSERM-484 · Laboratoire de Pharmacognosie et Biotechnologies · Faculté de Pharmacie, Université d'Auvergne · 63001 Clermont-Ferrand · France · Phone/Fax: +33-4-7317-8026 · E-mail: Chantal.Barthomeuf@u-clermont1.fr

Received July 20, 2007 · **Revised** October 4, 2007 · **Accepted** October 19, 2007

Bibliography
Planta Med 2007; 73: 1563–1567 © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2007-993754 · Published online December 11, 2007
ISSN 0032-0943

(bicyclic lignan) and diphyllin (arylnaphthalene lignan), both isolated from *H. perforatum* Kar. et Kir., a species endemic to Uzbekistan. The structures are presented in Fig. **1**.

Eudesmin has previously been extracted from *H. acutifolium* [2]. It has shown weak toxicity in mice [3] and has the capacity to inhibit tumour necrosis factor-alpha (TNF-α) production and T cell proliferation without toxicity for human macrophages [4]. Diphyllin has been isolated from *H. alberti-regelii, H. bucharicum,* and *H. buxbaumii* [5], [6] and, from various highly poisonous plants of the Acanthaceae family [7], [8], [9]. It exhibits antileishmanial activity [10] and growth-inhibitory effects on various cancer cell lines, including KB (cervical), HCT116 (colon), MCF-7 (breast) and LoVo (colon) carcinoma cells [9]. In this study, we examined if eudesmin and diphyllin inhibit drug transport by P-glycoprotein (Pgp, ABCB1, MDR1 protein), a drug efflux pump involved in the development of multidrug resistance [MDR] and poor response to chemotherapy in various malignancies [11]. To assess their potential in cancer therapy, we further compared the selectivity for cancer cells and the tumoricidal activity of these lignans with those of etoposide (4′-demethylepipodophyllotoxin, 4,6-*O*-ethylidene-β-D-glucopyranoside) and podophyllotoxin on healthy cells including human primary fibroblasts, and on a set of human solid cancer lines including a resistant breast carcinoma line overexpressing Pgp.

Material and Methods

Cells and chemicals

Human primary fibroblasts were purchased from Biopredic International (Rennes, France). Human MCF7 breast adenocarcinoma, A549 non-small cell lung carcinoma, PC3 androgen-resistant prostate carcinoma, DLD1 colon adenocarcinoma, PA1 ovary teratocarcinoma were obtained from the European Collection of Cell cultures (ECACC; Salibury, UK). Resistant MCF7/Dox resulted from 1 year continuous exposure of MCF7 cells to doxorubicine [Dox]. Western blots with specific monoclonal antibodies have shown that this resistant tumour line expresses the multidrug transporters P-glycoprotein (Pgp, ABCB1, MDR1 protein) and multidrug-resistant protein-1 (MRP-1, ABCC1) but not breast cancer-resistance protein (BCRP, ABCG2). Flow cytometry analysis has established the MDR1/MRP1 protein expression ratio as 5/1.3 (Barthomeuf, unpublished data). MDR1-transfected Madin-Darby canine kidney (MDCK-MDR1) cells were a gift of Amanda Yancy (AstraZeneca Pharmaceuticals). This cell line expresses the human MDR1 gene encoding Pgp. M4Beu human malignant melanoma were received from the laboratory of Dr. J. F. Doré (INSERM Unit 218, Lyon, France). Cells were maintained at 37 °C under 5% CO_2 in Eagle's minimum essential medium (Gibco-BRL; Paisley, Scotland) supplemented with 10% foetal calf serum (FBS; Bio West; Nuaillé, France), vitamins, amino acids, and antibiotics (all from Gibco™, Invitrogen; Cergy-Pontoise, France) as described in [12].

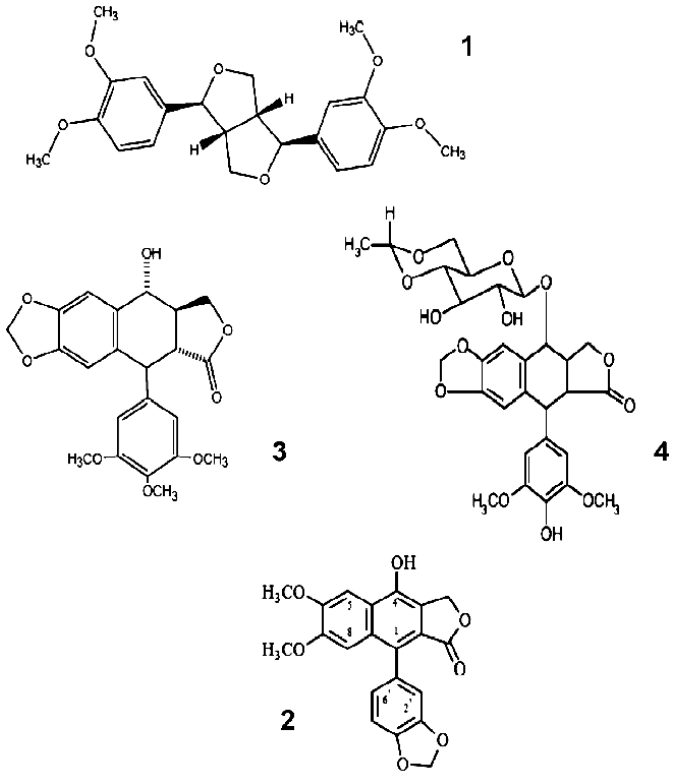

Fig. **1** Structures of eudesmin (**1**), diphyllin (**2**), podophyllotoxin (**3**) and etoposide (**4**).

The furanocoumarin cnidiadin was purified as previously reported [13]. PSC833 was a gift of Novartis Pharma. Other chemicals were from Sigma (St Louis, MO, USA).

Plant material

Leaves and roots of *H. perforatum* Kar. et Kir. (Rutaceae) were collected at the flowering stage and were identified by Dr. A. M. Nigmatullaev, Laboratory of Medicinal Plants, S. Yu. Yunusov Institute of the Chemistry of Plant Substances. Voucher specimens (2017 and 2021) have been deposited at the Institute's Herbarium.

Eudesmin and diphyllin isolation

Diphyllin ($C_{21}H_{16}O_{70}$, 4-hydroxy-3-hydroxymethyl-6,7-dimethoxy-1-(3′,4′-methylenedioxyphenyl)-2-naphthoic acid) and eudesmin ($C_{22}H_{26}O_6$, 2,6-bis-(3′,4′-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane) were respectively isolated from *H. perforatum* Kar. et Kir. roots and the leaves according to established procedures [2], [5]. They were formally identified at the Yunusov Institute (Chemistry of Plant Substances, Tashkent) by means of optical rotation ($[\alpha]_D^{20}$), UV, IR, EI-mass and ^{1}H-NMR (400 MHz with TMS as internal standard and $CDCl_3$ as solvent) spectroscopy. All data were in agreement with those reported in the literature for authentic compounds [2], [5], [13].

Cytotoxicity assay

The cytotoxicity of each treatment was determined after a 48-h exposure to each lignan (8 concentrations) by the resazurin reduction test (RTT) which determines the metabolic activity of cultures as the amount of fluorescent resorufin produced by living cells. Assays were carried out according to [12]. Etoposide and podophyllotoxine (both from Sigma) served as positive controls and the solvent (DMSO 0.5%) as negative control.

Rhodamine-123 (R-123) accumulation

Tests were carried out on MDCK-MDR1 cells or MCF7/Dox (150,000 cells/well) cultured in 24-well plates. R-123 uptake was determined by fluorimetry at 485 nm (λ_{ex})/538 nm (λ_{em})

120 min after cell exposure to R-123 and solvent or tested compounds as described in [14]. Cyclosporin A (CsA), PSC833 or cnidiadin or both (Aventis; West Laval , Canada) were used as positive controls and the solvent as negative control.

[^3H]-Vinblastine ([^3H]-VBL) accumulation in MDCK-MDR1 cells

[^3H]-VBL was determined by scintillation counting 120 min after cell exposure to [^3H]-VBL (0.23 Ci) in the presence or absence of tested compounds according to [15]. CsA and cnidiadin were used as positive controls and the solvent as negative control.

Statistics

Statistical analysis were done by means of Student's t-test. $P < 0.05$ was considered as significant.

Results

At first we compared the antiproliferative effects of a 48-h treatment by eudesmin (0 – 100 M), diphyllin (0 – 100 M), etoposide (0 – 100 M) or podophyllotoxin (0 – 10 M) on DLD1 (colon), MCF7 (breast), PC3 (prostate), PA1 (ovary) and A549 (lung) carcinoma cells, on M4Beu human malignant melanoma cells and on human primary fibroblasts. Because etoposide is effluxed by Pgp, the lignans were also tested on resistant MCF7/Dox, a MDR1/MRP1-positive doxorubicin-resistant breast carcinoma subline. Additional experiments were conducted on MDCK-MDR1 cells, a mammal cell line which selectively overexpress Pgp. Tests were carried out on exponentially growing cells cultured in the presence of 10% FBS. Data evidenced significant differences between the 4 lignans (Table **1**).

In agreement with toxicity data in white mice [LD_{50}: 278 mg/kg (*i.v.*) and 1900 mg/kg (per os)] [8], the bicyclic lignan eudesmin exhibited very low toxicity (IC_{50} > 100 M) on healthy lines (normal fibroblasts, MDCK and MDCK-MDR1 cells). It also exhibited very low (IC_{50} > 100 M) tumoricidal effects. In contrast, all arylnaphthalene lignans displayed marked tumoricidal activity, in particular on highly proliferative human PA1 ovary carcinoma cells. They, however, differed by their level and pattern of cytotoxicity. Diphyllin was markedly less toxic than podophyllotoxin (IC_{50}: 13 – 61 nM), but exhibited tumoricidal effects close to those of etoposide, including Pgp-overexpressing cells. The main differences were a lower efficacy on PA1 cells (IC_{50}: 1.7 M vs. < 0.2 M) and a higher potency (IC_{50}: 1.9 M vs. 16 M) on M4Beu malignant melanoma cells. Unfortunately, diphyllin was also 65-fold more toxic than etoposide on human primary fibroblasts and conversely to this drug exhibited a negative cytotoxicity index on all cancer cells. The lower efficacy of diphyllin in MDCK/MDR1 cells demonstrated that this arylnaphthalene lignan is effluxed and consequently interacts with Pgp.

Cells overexpressing Pgp are characterised by a lower capacity to accumulate and retain certain dyes [including rhodamine-123 (R-123)] and anticancer drugs (including doxorubicin, etoposide, *Vinca* alkaloids, taxanes). To determine if diphyllin and eudesmin may reverse multidrug resistance, we examined how a 120-min exposure to these lignans affected R-123 and [^3H]-vinblastine ([^3H]-VBL) accumulation in MDCK/MDR1 and MDCK cells. Tests were carried out vs. cnidiadin (a competive inhibitor of Pgp transport [14]), cyclosporin A (CsA) or PSC833.

Exposure of resistant MDCK/MDR1 cells to 100 M diphyllin, eudesmin and cnidiadin increased the R-123 uptake by, respectively, 230 ± 25%, 147 ± 17% and 610 ± 19% (Fig. **2A**). As expected, any R-123 accumulation was observed in sensitive MDCK cells exposed to these chemicals (not shown). The background uptake was not affected when the cells were exposed to 10 M eudesmin or cnidiadin, but was increased by 21 ± 7%, 550 ± 10% and 630 ± 16%, after exposure to 10 M of diphyllin, CsA or SDZ-PSC833, respectively (Fig. **2A**). Note that exposure to 10 M diphyllin, eudesmin and CsA also promoted R-123 accumulation in MCF7/Dox cells. In this resistant human tumour cell line, the amount of R-123 accumulated by each lignan was higher than in MDCK/MDR1. However, the background uptake was also more importantly increased by diphyllin (87 ± 17%) than by eudesmin (11 ± 7%) (data not presented).

Table **1** Cytotoxicity pattern of eudesmin, diphyllin, epipodophyllotoxin and etoposide

Cell line	*Etoposide IC_{50} (M)*	*Podophyllotoxin IC_{50} (M)*	*Diphyllin IC_{50} (M)*	*Eudesmin IC_{50} (M)*
Primary human fibroblasts	> 100	13.0 ± 0.0	1.5 ± 0.6	> 100
PC3 (Prostate carcinoma)	20.0 ± 1.1	19.0 ± 0.0	18.0 ± 3.9	> 100
MCF7 (Breast carcinoma)	> 50	61.0 ±0.0	54.0 ± 2.9	> 100
MCF7/Dox*	> 100	ND	> 100	> 100
PA1 (Ovary carcinoma)	< 0.2	13.0 ± 0.0	1.7 ± 0.7	> 100
DLD1 (Colon carcinoma)	7.0 ± 2.9	29.0 ± 0.5 × 10^{-3}	4.9 ± 1.1	> 100
A549 (Lung carcinoma)	1.0 ± 0.2	15.0 ± 0.0	2.1 ± 0.5	> 100
M4Beu (Malignant melanoma)	16.0 ± 4.9	18.0 ± 0.0	1.9 ± 0.5	> 100
MDCK	ND	ND	0.9 ± 0.2	> 100
MDCK-MDR1*	ND	ND	3.1 ± 0.4	> 100

The cytotoxicity was measured after a 48-h exposure to each chemical by means of the resazurin reduction test (RRT) which measures the amount of fluorescent resofurin produced by reductases from living cells. Tests were carried out in triplicate at 8 concentrations. The solvent (DMSO 0.5%) served as negative control. The amount of resorufin present in treated and control wells was assessed at 530 nm (λ_{ex})/590 nm (λ_{em}). IC_{50} (mean ± S.D) values were calculated with the *ALLFIT* program and were expressed in M. The cell-line(s) exhibiting the highest susceptibility to each lignan appears in bold.

Resistant cell lines: MCF7/Dox (human breast cancer line resistant to doxorubicine), MDCK-MDR1 (Madin-Darby canine kidney cells expressing the human MDR1 gene encoding Pgp).

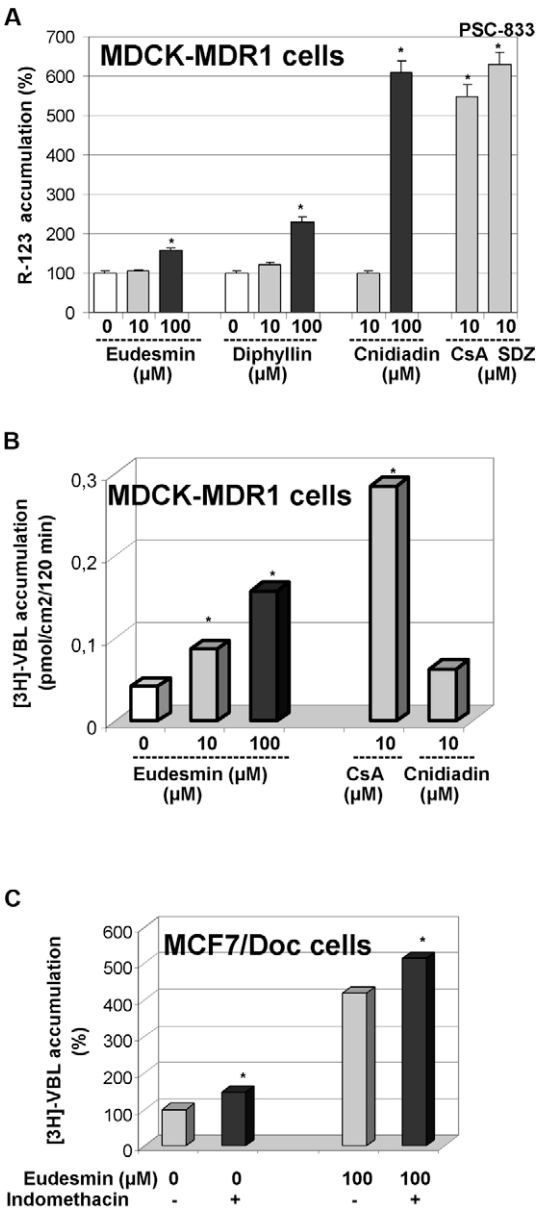

Fig. **2** Enhancement of dye and drug uptake in Pgp-overexpressing cells exposed to diphyllin or eudesmin. **A**) Enhancement of rhodamine-123 (R-123) uptake in MDCK-MDR1 cells exposed to the solvent (control), to 10 M or 100 M of eudesmin, diphyllin or cnidiadin or to 10 M of cyclosporin A (CsA) or SDZ-PSC833. **B**) Accumulation of [^3H]-vinblastine ([^{3}H]-VBL) in MDCK-MDR1 cells exposed to the solvent or eudesmin (10, 100 M), cnidiadin (10, 100 M) or CsA (10 M). **C**) Accumulation of [^3H]-VBL in MCF7/Dox cells exposed to the MRP inhibitor indomethacin (10 M) in the absence or the presence of eudesmin (100 M). R-123 and [^3H]-VBL uptakes were measured 120 min after cell exposure to the specified concentrations of each chemical. Data are the mean of at least 2 independent experiments performed in duplicate. They are expressed in percentage (R-123) or in pmol/cm^2/120 min ([^3H]-VBL). * implicates significant differences as compared to control values ($p < 0.05$, Student's t test).

It can be deduced from the R-123 data that the arylnaphthalene-derived skeleton provides a higher reversal effect than the bis-dimethoxyphenyldioxabicyclooctane one. However, the high toxicity of diphyllin for healthy cells precludes its clinical use. Accordingly, this lignan was not further tested. Additional tests were carried out on eudesmin which is weakly toxic and displays clinically relevant biological effects [3], [4]. To determine if this bicyclic lignan may affect the bioavailability and clinical potency of anticancer drugs in Pgp-overexpressing cells, we further examined how it affected the accumulation of [^3H]-vinblastine ([^3H]-VBL) in MDCK-MDR1 cells. Significant drug accumulation (+ 187 ± 17 %) was observed 120 min after exposure to 10 M eudesmin. Exposure to 100 M eudesmin enhanced drug accumulation to 369 ± 21 % (Fig. **2B**).

[^3H]-VBL is transported by both Pgp and MRP transporters. To determine how the reversal effect of eudesmin was specific to Pgp, we further examined whether the MRP inhibitor indomethacin (10 M) affected eudesmin-mediated accumulation of [^3H]-VBL in MCF7/Dox cells. When the cells were exposed to indomethacin in the absence of eudesmin, the background uptake was increased by 47 ± 7 %, confirming that MCF7/Dox cells express MRP transporters. That exposure to the eudesmin/diphyllin combination promoted slighly higher accumulation of [^3H]-VBL (475 % ± 13 %) than eudesmin singly (420 ± 15 %) supports the hypothesis that eudesmin has only a broad specificity for Pgp (Fig. **2C**).

Discussion

This study reports the first extraction of eudesmin from *H. perforatum* Kar. et Kir endemic to Uzbekistan and the first extraction of diphyllin from the roots of this species. Since diphyllin has previously been extracted from the *H. perforatum* seeds [3], this species produces lignans in all plant organs and, interestingly, produces lignans belonging to different structural groups. Cytotoxicity data have shown that although diphyllin has no value for cancer treatment, it is highly cytotoxic. Since *H. perforatum* roots contain the same diphyllin content (0.1 % dry mass) as those of *H. bucharicum* [5], diphyllin is certainly for the most part responsible for the high toxicity of their root extracts.

Pgp and MRP are the major causes of cancer chemotherapy failure. Analysis of dye and drug uptake in MDR1-transfected MDCK cells provide the first demonstration that eudesmin, a bicyclic lignan, inhibits Pgp-mediated drug efflux. Data in MCF7/Dox, a resistant human tumour line expressing both Pgp and MRP protein, suggest that it has only a broad specificity for Pgp and may reverse both MDR1- and MRP-mediated resistance to drug. However, this latter point remains to be evidenced. The reversal activity of eudesmin is certainly insufficient for clinical application. However, its capacity to accumulate [^3H]-VBL in MDCK-MDR1 cells supports the hypothesis that this non-toxic lignan or, eudesmin-rich extracts, may positively affect the bioavailability, and consequently the therapeutic potency, of anticancer drugs in Pgp-overexpressing cells.

Cytotoxicity data unambiguously demonstrate that the unglycosylated arylnaphthalene lignans podophyllotoxin and diphyllin have no value as anticancer drugs. It has been reported that cleistanthin A, a diphyllin glycoside extracted from *Cleistanthus collinus* (Euphorbiaceae), is more toxic for cancer than normal cells [16]. Together with our data, this information provides a new demonstration that glycans play a crucial role in the specificity of arylnaphthalene lignans for tumor cells. Cleistanthin A is claimed to increase the life span of mice with S-180 sarcoma to a

similar degree as cisplatin or etoposide, with the advantage of lower toxicity. Its anti-tumour effects have been attributed to inhibition of DNA synthesis, cell-cycle blockade, induction of apoptosis and, down-regulation of matrix metalloproteinase-9 activity [16], [17]. Biologically active diphyllin glycosides have also been extracted from *Justicia procumbens* [18] and from certain *Haplophyllum* species [19] or derived-shoot cultures [20]. Yet, the value of diphyllin as raw material for the hemisynthesis of anticancer drugs is very limited. However, if the clinical value of cleistanthin A (or other diphyllin glycosides) is demonstrated, this relatively abundant lignan might have a possible usefulness in this application.

Acknowledgements

The authors wish to thank INTAS (International association for the promotion of co-operation with scientists from the New Independent States of the former Soviet Union) for its support and partial financial contribution to this work. The work was supported by INSERM and by grants from the Natural Sciences and Engineering Council of Canada to R Beliveau.

References

[1] Ibn Sina AA (Avicenna). The handbook of medical sciences. Selected parts. Tashkent: FAN; 1985: 264 – 5.

[2] Razzakova DM, Bessonova IA, Yunusov SYu. Eudesmin, a lignan from *Haplophyllum acutifolium* and *H. perforatum*. Chem Nat Compounds 1972; 8: 646 – 7.

[3] Akhmedkhodzhaeva Kh S, Kurmukov AG. Some pharmacological properties of eudesmin. Reports Uzb Acad Sci 1975; 1: 34 – 5.

[4] Cho JY, Yoo ES, Baik KU, Park MH. Eudesmin inhibits tumor necrosis factor-alpha production and T cell proliferation. Arch Pharm Res 1999; 22: 348 – 53.

[5] Nesmelova EF, Razakova DM, Akhmedjanova VI, Bessonova IA. Diphyllin from *Haplophyllum alberti-regelii, H. bucharicum*, and *H. perforatum*. Chem Nat Compounds 1983; 19: 608 – 9.

[6] Al-Abed Y, Abu-Zarga M, Sabri S, Atta-Ur-Rahman, Voelter W. A arylnaphthalene lignan from *Haplophyllum buxbaumii*. Phytochemistry 1998; 49: 1779 – 81.

[7] Okigawa M, Maeda T, Kawano N. The isolation and structure of three new lignans from *Justica procumbens* Linn. var. *leucantha* Honda. Tetrahedron 1970; 26: 4301 – 5.

[8] Fukamiya N, Lee KH. Antitumor agents, 81. Justicidin-A and diphyllin, two cytotoxic principles from *Justicia procumbens*. J Nat Prod 1986; 49: 348 – 50.

[9] Susplugas S, Hung NV, Bignon J, Thoison O, Kruczynski A, Sevenet T et al. Cytotoxic arylnaphthalene lignans from a Vietnamese acanthaceae, *Justicia patentiflora*. J Nat Prod 2005; 68: 734 – 8.

[10] Di Giorgio C, Delmas F, Akhmedjanova V, Ollivier E, Bessonova I, Riad E et al. *In vitro* antileishmanial activity of diphyllin isolated from *Haplophyllum bucharicum*. Planta Med 2005; 71: 366 – 9.

[11] Leonard GD, Fojo T, Bates SE. The role of ABC transporters in clinical practice. Oncologist 2003; 8: 11 – 24.

[12] Barthomeuf C, Debiton E, Mshvildadze V, Kemertelidze E, Balansard G. *In vitro* activity of hederacolchiside A1 compared with other saponins from *Hedera colchica* against the proliferation of human carcinoma and melanoma cells. Planta Med 2002; 68: 672 – 5.

[13] Govindachari TR, Sathe SS, Viswanathan N, Pai BR, Srinivasan M. Revised structures of diphyllin and justicidin A. Tetrahedron Lett 1967; 36: 3517 – 9.

[14] Barthomeuf C, Grassi J, Demeule M, Fournier C, Boivin D, Beliveau R. Inhibition of P-glycoprotein transport function and, reversion of MDR1 multidrug resistance by cnidiadin. Cancer Chemother Pharmacol 2005; 56: 173 – 81.

[15] Barthomeuf C, Demeule M, Grassi M, Saidkhodjaev A, Beliveau R. Conferone from *Ferula schtschurowskiana* enhances vinblastine cytotoxicity in MDCK-MDR1 cells by competively inhibiting P-glycoprotein transport function. Planta Med 2006; 72: 634 – 9.

[16] Pradheepkumar CP, Shanmugam G. Anticancer potential of cleistanthin A isolated from the tropical plant *Cleistanthus collinus*. Oncol Res 1999; 11: 225 – 32.

[17] Meenakshi J, Shanmugam G. Inhibition of matrix metalloproteinase-9 (MMP-9) activity by cleistanthin A, a diphyllin glycoside from *Cleistanthus collinus*. Drug Dev Res 2000; 50: 93 – 4.

[18] Day SH, Lin YC, Tsai ML, Tsao LT, Ko HH, Chung MI et al. Potent cytotoxic lignans from *Justicia procumbens* and their effects on nitric oxide and tumor necrosis factor-alpha production in mouse macrophages. J Nat Prod 2002; 65: 379 – 81.

[19] Prieto JM, Giner RM, Recio MC, Schinella G, Manez S, Rios JL. Diphyllin acetylapioside, a 5-lipoxygenase inhibitor from *Haplophyllum hispanicum*. Planta Med 2002; 68: 359 – 60.

[20] Innocenti G, Puricelli L, Piacente S, Caniato R, Filippini R, Cappelletti EM. Patavine, a new arylnaphthalene lignan glycoside from shoot cultures of *Haplophyllum patavinum*. Chem Pharm Bull 2002; 50: 844 – 6.