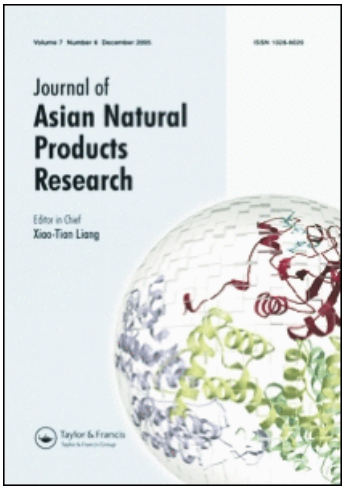
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

The enhancement of vincristine cytotoxicity by combination with feselol

Samaneh Mollazadeh[a]; Maryam M. Matin[ab]; Mehrdad Iranshahi[c]; Ahmad Reza Bahrami[ab]; Vajiheh Neshati[a]; Fatemeh Behnam-Rassouli[a]
[a] Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran [b] Cell and Molecular Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran [c] Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Online publication date: 13 July 2010

To cite this Article Mollazadeh, Samaneh , Matin, Maryam M. , Iranshahi, Mehrdad , Bahrami, Ahmad Reza , Neshati, Vajiheh and Behnam-Rassouli, Fatemeh(2010) 'The enhancement of vincristine cytotoxicity by combination with feselol', Journal of Asian Natural Products Research, 12: 7, 569 — 575
To link to this Article: DOI: 10.1080/10286020.2010.485565
URL: http://dx.doi.org/10.1080/10286020.2010.485565

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research
Vol. 12, No. 7, July 2010, 569–575

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The enhancement of vincristine cytotoxicity by combination with feselol

Samaneh Mollazadeh[a], Maryam M. Matin[ab]*, Mehrdad Iranshahi[c], Ahmad Reza Bahrami[ab], Vajiheh Neshati[a] and Fatemeh Behnam-Rassouli[a]

[a]*Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran;* [b]*Cell and Molecular Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran;* [c]*Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

(*Received 13 December 2009; final version received 11 April 2010*)

Urinary bladder cancer is one of the most common cancers worldwide. Human transitional cell carcinoma (TCC) cells are epithelial-like adherent cells originally established from a primary bladder carcinoma. Studies have shown that TCC cells are resistant to some chemotherapeutic agents such as vincristine (VCR). In the present study, the effect of feselol, a sesquiterpene coumarin isolated from the fruits of *Ferula badrakema*, was investigated on VCR effectiveness. Our results demonstrated that feselol itself did not have any cytotoxic effect on TCC cells. In order to check its combinatorial effects, TCC cells were exposed to various combined concentrations of feselol and VCR. Then, morphological changes were monitored and cytotoxicity was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay for three consequent days. Results showed that the combination of 40 μg/ml VCR with 16 μg/ml feselol increased the cytotoxicity of VCR by 28.32% after 48 h. This effect might be due to inhibition of P-glycoprotein in TCC cells by feselol.

Keywords: *Ferula badrakema*; sesquiterpene coumarin; feselol; vincristine; TCC cells

Downloaded At: 18:23 22 January 2011

1. Introduction

There are various types of bladder cancer; superficial tumors tend to recur but never progress and invade, while other types eventually progress to invasive cancers [1]. Besides surgery and radiotherapy, systemic chemotherapy is used as a complementary treatment for this kind of cancer [2]. Transitional cell carcinoma (TCC) is the main histological form of bladder cancer, which is most prevalent in Western and industrialized countries [3].

Vincristine (VCR) is a vinca alkaloid derived from *Catharanthus roseus* [4]. It was shown that TCC cells are resistant to VCR by over-expressing *MDR1* gene [5,6].

The exclusively old world genus *Ferula* belongs to the family Umbelliferae with about 130 species distributed throughout central Asia, especially in countries such as Iran [7]. This genus is well documented as a good source of biologically active compounds including coumarins and sesquiterpene derivatives [8–10]. *Ferula badrakema* [11], similar to other species of genus *Ferula* is a rich source of sesquiterpene coumarins [12]. It was shown that some drimane-type

*Corresponding author. Email: matin@um.ac.ir

ISSN 1028-6020 print/ISSN 1477-2213 online
© 2010 Taylor & Francis
DOI: 10.1080/10286020.2010.485565
http://www.informaworld.com

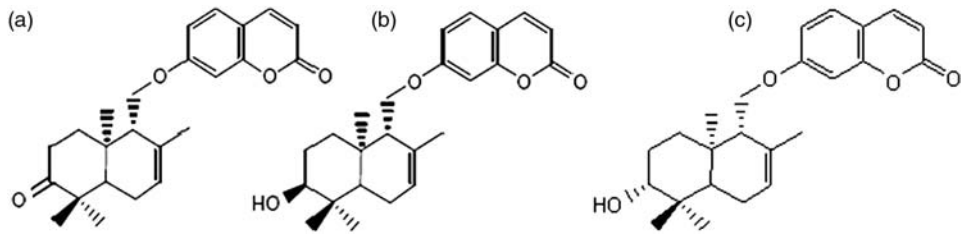


Figure 1. The chemical structures of (a) conferone, (b) mogoltacin, and (c) feselol, drimane-type sesquiterpene coumarins isolated from *Ferula* species.

sesquiterpene coumarins such as conferone and mogoltacin (Figure 1) can increase drug accumulation and effectiveness by P-glycoprotein (P-gp) inhibition [13–15].

In the present study, the effects of feselol, which is a drimane-type sesquiterpene coumarin isolated from the fruits of *F. badrakema*, on VCR activity was investigated in TCC cells.

2. Results and discussion

In order to test the effects of feselol on VCR cytotoxicity, it was first necessary to analyze the effects of feselol itself on TCC cells. To do so, cells were exposed to different concentrations of feselol (8, 16, 32, and 64 μg/ml) for 24, 48, and 72 h. Analyzing the cell survival by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay showed that in comparison with equivalent amounts of dimethylsulfoxide (DMSO) (0.4, 0.8, 1.6, and 3.2%) as control groups, feselol did not have any cytotoxic effect on TCC cells. In order to determine the half maximal inhibitory concentration (IC_{50}) value of VCR on TCC cells, cells were treated with different increasing concentrations of VCR (25, 50, 100, 250 μg/ml) for three consequent days. The IC_{50} of VCR was calculated as 49 μg/ml on these neoplastic cells using MTT assay (data not shown). For analyzing the effect of feselol on VCR activity, TCC cells were cultured in the presence of various combining concentrations of feselol (8, 16, 32, and 64 μg/ml) and VCR (30, 40, and 50 μg/ml), close to and less than its IC_{50} value. Then, cell viability and morphological changes were monitored during the 72 h following drug administration. Evaluation of cell viability by one-way analysis of variance (ANOVA) and the Tukey test showed that there were significant differences between test (feselol–VCR) and control (DMSO–VCR) groups, especially 48 h after drug administration (Table 1). Using the least significant difference (LSD) test showed that 16 μg/ml feselol could significantly ($P < 0.001$) enhance the cytotoxicity of 40 μg/ml VCR by 28.32% after 48 h, compared with other combined concentrations during 24, 48, and 72 h (Figure 2; Table 2).

The effects of feselol on VCR cytotoxicity were also confirmed by morphological observations. Cells treated with different concentrations of feselol–VCR and DMSO–VCR were monitored during the 72 h after drug administration. The most obvious changes were observed after 48 h of co-culture of TCC cells with the combination of 16 μg/ml feselol and 40 μg/ml VCR. Cells became rounded and deformed with granulated cytoplasm, and the cell number was significantly decreased in comparison with controls (Figure 3).

One of the main problems in the clinical treatment of cancer is the resistance of tumor cells to chemotherapeutic agents. This phenomena, which is known as multidrug resistance (MDR), is responsible for the poor clinical outcome of

Downloaded At: 18:23 22 January 2011

Downloaded At: 18:23 22 January 2011

Table 1. Comparison of cell survival percentage in 36 groups of treatments; combination of 8, 16, 32, and 64 μg/ml feselol with 30, 40, and 50 μg/ml VCR and their controls of DMSO (mean ± SEM).

Time (h)	VCR (μg/ml)	0.4% DMSO	Feselol 8 μg/ml	0.8% DMSO	Feselol 16 μg/ml	1.6% DMSO	Feselol 32 μg/ml	3.2% DMSO	Feselol 64 μg/ml
24	30	90 ± 0.01	85 ± 0.00	99 ± 0.02	75 ± 0.01*	93 ± 0.01	72 ± 0.01*	102 ± 0.01	78 ± 0.00*
	40	87 ± 0.01	83 ± 0.03	95 ± 0.00	77 ± 0.00*	93 ± 0.01	74 ± 0.01*	98 ± 0.02	81 ± 0.02*
	50	86 ± 0.00	82 ± 0.00	93 ± 0.02	77 ± 0.00*	89 ± 0.00	75 ± 0.01*	100 ± 0.03	80 ± 0.00*
48	30	79 ± 0.01	77 ± 0.01	90 ± 0.03	74 ± 0.01*	91 ± 0.00	74 ± 0.01*	104 ± 0.04	82 ± 0.01*
	40	80 ± 0.02	79 ± 0.00	94 ± 0.01	69 ± 0.00*	85 ± 0.00	76 ± 0.01*	105 ± 0.01	82 ± 0.01*
	50	83 ± 0.01	78 ± 0.03	90 ± 0.00	71 ± 0.01*	84 ± 0.01	70 ± 0.00*	108 ± 0.01	83 ± 0.00*
72	30	77 ± 0.00	76 ± 0.01	80 ± 0.01	72 ± 0.00*	80 ± 0.00	73 ± 0.01	95 ± 0.02	91 ± 0.01
	40	79 ± 0.01	78 ± 0.00	83 ± 0.00	67 ± 0.00*	77 ± 0.02	76 ± 0.00	100 ± 0.02	90 ± 0.00
	50	75 ± 0.02	73 ± 0.00	80 ± 0.02	72 ± 0.01*	76 ± 0.01	70 ± 0.00	99 ± 0.02	93 ± 0.01

Note: The results of the Tukey test compared the effects of all feselol and VCR combined concentrations with their DMSO analogues. *$P < 0.05$, significant difference between test and control groups.

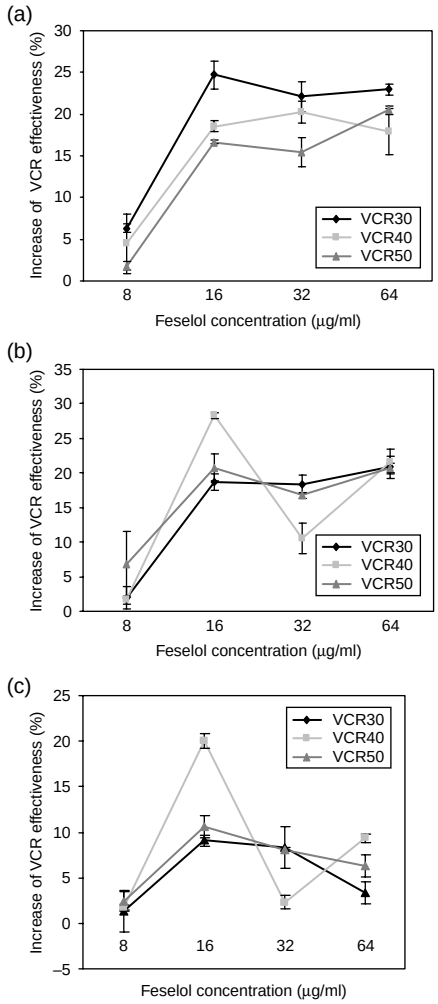


Figure 2. The dose response curves of different combined concentrations of feselol and VCR (a) 24, (b) 48, and (c) 72 h after cell treatment.

chemotherapy [16]. Classic MDR is mainly mediated by overexpression of P-gp, a product of human *MDR1* gene, which is an ATP-dependent drug-efflux membrane transporter [17,18]. Studies have revealed that chemotherapeutic agents could enhance drug sensitivity in resistant cells using chemosensitizers, MDR reversal agents, modulators or converters [16]. Modulating P-gp activity by a number of agents including verapamil (classical P-gp modulator) and cyclosporine A results in drug accumulation and activity in resistant cells [18]. However, effective MDR modulators have not been used clinically because of their toxicity or side effects [18]. Reports indicated that intracellular accumulation of VCR was decreased by P-gp-mediated drug resistance [19]. It has also been shown that TCC cells are resistant to vinca alkaloids [5], by overexpression of *MDR1* gene [6].

In the present study, the effects of feselol, a drimane-type sesquiterpene coumarin, was investigated on VCR activity in TCC cells. Our results demonstrated that the combination of 16 μg/ml feselol with 40 μg/ml VCR increased the cytotoxicity of VCR by 28.32%, after 48 h of drug administration. Moreover, higher concentrations of feselol did not enhance VCR cytotoxicity. This might be due to the increasing amounts of DMSO, which can prevent the effects of feselol to be observed. These results indicate that feselol can enhance VCR activity presumably by inhibiting P-gp, and thus increasing the sensitivity of TCC cells to VCR. Furthermore, it was shown that 8, 16, 32, and 64 μg/ml of feselol in single use did not have any toxic effects on the cells. Therefore, feselol can be considered as a good candidate to reverse MDR in clinical studies.

It has been shown that cnidiadin, as a furanocoumarin, is capable of inhibiting P-gp-mediated drug efflux and increasing vinca alkaloids toxicity *in vitro* [20]. It has also been reported that sesquiterpene coumarins from *Ferula* genus can act as reversal agents; there is evidence that conferone, a sesquiterpene isolated from *Ferula schtschurowskiana*, can increase intracellular accumulation of vinblastine by P-gp inhibition [13]. Furthermore, some sesquiterpenes from the leaves of *Zinowiewia costaricensis* showed higher effectiveness than verapamil when used in combination with vinblastine [21]. Investigators have shown that sesquiterpene coumarins, as reversal agents, can directly react with the intermembrane domain of

Downloaded At: 18:23 22 January 2011

Table 2. The increased percentage of VCR cytotoxicity in TCC cells exposed to different combined concentrations of feselol and VCR (mean ± SEM).

Time (h)	VCR (μg/ml)	Feselol 8 μg/ml	Feselol 16 μg/ml	Feselol 32 μg/ml	Feselol 64 μg/ml
24	30	6.28 ± 0.50	24.75 ± 1.68	22.09 ± 1.79	22.94 ± 0.67
	40	4.45 ± 3.57	18.52 ± 0.66	20.25 ± 1.25	17.90 ± 2.77
	50	1.81 ± 0.54	16.63 ± 0.22	15.45 ± 1.78	20.50 ± 0.52
48	30	1.89 ± 1.61	18.64 ± 1.19	18.41 ± 1.35	20.85 ± 1.60
	40	1.64 ± 0.57	*28.32 ± 0.39	10.56 ± 2.21	21.63 ± 1.80
	50	6.84 ± 4.77	20.77 ± 1.96	16.75 ± 0.45	20.74 ± 0.63
72	30	1.34 ± 2.26	9.1 ± 0.58	8.31 ± 2.3	3.31 ± 1.19
	40	1.72 ± 0.3	20 ± 0.79	2.28 ± 0.73	9.37 ± 0.48
	50	2.38 ± 1.08	10.59 ± 1.23	8.11 ± 0.26	6.29 ± 1.16

Note: The results of the LSD test compared the effects of all feselol concentrations on all VCR cytotoxicities over 3 days. As shown in the table, 48 h after combination of 40 μg/ml VCR with 16 μg/ml feselol, the cytotoxicity of VCR increased by 28.32% (*).

Downloaded At: 18:23 22 January 2011

P-gp [13]. More studies on other cell lines overexpressing P-gp are required to determine if feselol and other sesquiterpene coumarins specifically inhibit P-gp transport.

In summary, as the main cause of failure in cancer treatment is acquired resistance to anticancer agents in malignant tumors, it is important to find new chemical agents that can provoke tumors to become less resistant during treatment. Our data raise the possibility that feselol may target P-gp, and since it is not toxic on TCC cells, it might be useful to reverse MDR in clinical trials.

3. Experimental

3.1 Feselol preparation

Fruits of *F. badrakema* were collected from Hezarmasjed mountains, north east of Iran, in August 2005, and identified by Ferdowsi University of Mashhad Herbarium. The air-dried fruits (500 g) were grounded into powder, defatted by petroleum ether and extracted exhaustively by maceration with dichloromethane at room temperature. After filtration, the extract was concentrated under vacuum to yield 20 g of a brown residue. Part of the extract (15 g) was subjected to column chromatography on silica gel (5× 50 cm) using petroleum ether with increasing volumes of acetone [petroleum ether–Me_2CO (20:1), (15:1), (10:1), (9:1), (8:1), (7:1), (6:1), (5:1), (4:1), (3:1), (2:1), (1:1), and (0:1)]. The fractions were compared by TLC (silica gel using petroleum ether–Me_2CO as solvent) and those giving similar spots were combined. Five fractions were finally obtained. Fraction 3, which was named feselol (Figure 1), was selected to identify its effects on VCR cytotoxicity. This fraction contained 48.5 mg feselol and its structure was confirmed by 1D and 2D NMR spectra as well as melting point. These spectroscopic data were in agreement with those previously described in the literature [22,23].

3.2 Culture and growth of TCC cells

Human bladder TCC cell line was obtained from Pasteur Institute (Tehran, Iran). The cells were grown in Dulbecco's modified Eagle's medium (Gibco, Scotland) supplemented with 10% fetal bovine serum (Gibco, Scotland) and incubated at 37°C in a humidified atmosphere in the presence of 10% CO_2. Cells were passaged using 0.25% trypsin-1 mM EDTA.

3.3 Preparation of various feselol concentrations

In order to prepare different concentrations of feselol (8, 16, 32, and 64 μg/ml), 2 mg

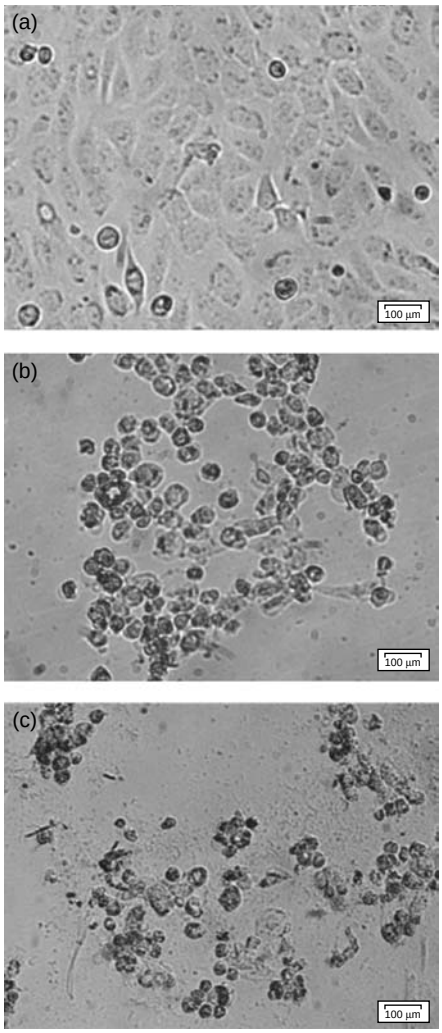


Figure 3. Morphological alterations of TCC cells 48 h after drug administration: (a) without any treatment, (b) treated with 0.8% DMSO and 40 μg/ml VCR, and (c) treated with 16 μg/ml feselol and 40 μg/ml VCR (×40).

of the powder was dissolved in 1 ml DMSO (Merck, Darmstadt, Germany). Various amounts of the obtained solution were then diluted by different volumes of culture medium before experiments. Since feselol was dissolved in DMSO, equivalent amounts of DMSO (0.4, 0.8, 1.6, and 3.2%, respectively) were used as control groups.

3.4 MTT cell viability assay

Evaluating cell viability in assays of cell proliferation and cytotoxicity was carried out by MTT test. The MTT assay is based on the cleavage of the yellow tetrazolium salt (MTT) to insoluble purple formazan crystals by mitochondrial dehydrogenases of active cells [24]. In summary, single cells were suspended in a 10 ml culture medium and seeded at a density of 8×10^3 cells per well in 96-well microplates (Falcon Becton-Dickinson, Ontario, Canada). The final volume of each well was adjusted to 200 μl by adding medium, and TCC cells were allowed to grow for 24–48 h. Then, cells were treated with various increasing concentrations of feselol (8, 16, 32, and 64 μg/ml) and VCR (25, 50, 100, 250 μg/ml) separately. After identifying the IC_{50} value of VCR, cells were exposed to different combined concentrations of feselol–VCR, as test groups, and equivalent DMSO–VCR, as control groups, for three days. IC_{50} was determined by the percentage of living cells at each dose. To assay cell viability, fresh MTT solution was prepared before each test by dissolving MTT (Sigma, Taufkirchen, Germany) in phosphate buffered saline to obtain the final concentration of 5 mg/ml. After adding 20 μl of MTT solution to each well, the plates were incubated at 37°C for 4 h. After this period the remaining MTT solution was removed and replaced with DMSO to solubilize formazan crystals (200 μl/well). The optical density of the resulting purple solution was spectrophotometrically measured at 570 nm using enzyme-linked immunosorbent assay (ELISA) plate reader. All experiments were performed in triplicates. An increase or decrease in cell number results in a concomitant alteration in the amount of formazan produced, indicating the cytotoxicity degree caused by the test material. Percentage of cell survival is expressed as (absorbance of treated cells per well/mean absorbance of control

Downloaded At: 18:23 22 January 2011

cells) × 100. The dose response curves were calculated over the range of combined concentrations of feselol–VCR and expressed as the mean percentage fraction of control ± standard error of means (SEM).

3.5 Morphological alterations

After treating TCC cells with different combined concentrations of feselol (8, 16, 32, and 64 μg/ml) and VCR (30, 40, and 50 μg/ml), morphological changes including cell shape, granulated cytoplasm, adhesiveness and population of the cells were monitored under the microscope during 24, 48, and 72 h after drug administration.

3.6 Statistical analysis

Statistical procedures were performed with SPSS, JMP4, and MSTAT softwares. Significant level was ascertained by one-way ANOVA, followed by the Tukey multiple comparison test. *P*-values of <0.05 in the Tukey test and <0.001 in the LSD test were considered to be significant. Results were expressed as mean ± SEM.

Downloaded At: 18:23 22 January 2011

Acknowledgements

The authors would like to thank Prof. Javad Behravan for his great advice. We are also grateful to Mrs Zeinab Neshati, Mr Porsa, and Mr Bagherpoure for their excellent technical assistance. This work was supported in part by a grant from Ferdowsi University of Mashhad.

References

[1] D. Chopin, R. Barei-Moniri, P. Maille, M.I. Frere-Belda, B. Muscatelli-Groux, and N. Merendino, *Pathology* **162**, 1139 (2003).
[2] C. Sternberg, A. Yagoda, H. Scher, R. Watson, N. Geller, and H. Herr, *Cancer* **64**, 2448 (1989).
[3] N. Sengupta, E. Siddiqui, and F.H. Mumtaz, *J. R. Soc. Promot. Health* **124**, 228 (2004).
[4] R. Dhamodharan, M.A. Jondan, D. Thrower, and L. Wilson, *Mol. Biol. Cell* **6**, 1215 (1995).
[5] D. Yu, S. Chang, and C.P. Ma, *Br. J. Urol.* **81**, 234 (1998).
[6] C. Wu, W. Zhang, J. Chang, Z. Zhao, G. Sun, and R. Han, *Chin. J. Clin. Oncol.* **3**, 191 (2006).
[7] D. French, *Ethnobothany of the Umbelliferae* (Academic Press, London, 1971).
[8] M. Iranshahi, P. Arfa, M. Ramezani, M.R. Jaafari, H. Sadeghian, C. Bassarello, S. Piacente, and C. Pizza, *Phytochemistry* **68**, 103 (2007).
[9] M. Iranshahi, F. Kalategi, R. Rezaee, A.R. Shahverdi, C. Ito, H. Furukawa, H. Tokuda, and M. Itoigawa, *Planta Med.* **74**, 147 (2008).
[10] C. Barthomeuf, S. Lim, M. Iranshai, and P. Chollet, *Phytomedicine* **15**, 103 (2008).
[11] K.H. Rechinger, J.M. Lemond, and I.C. Hedge, *Akademischev Druk-u. Verlagsanstalt, Graz* **162**, 269 (1994).
[12] T.V. Bukreeva and M.G. Pimenov, *Khim. Prir. Soedin.* **27**, 718 (1991).
[13] C. Barthomeuf, M. Demeule, J. Grassi, A. Saidkhodjaev, and R. Beliveau, *Planta Med.* **72**, 634 (2006).
[14] V. Neshati, M.M. Matin, M. Iranshahi, A.R. Bahrami, J. Behravan, S. Mollazadeh, and F. Behnam Rassouli, *Z. Naturforsch. C* **64**, 317 (2009).
[15] F. Behnam-Rassouli, M.M. Matin, M. Iranshahi, A.R. Bahrami, V. Neshati, S. Mollazadeh, and Z. Neshati, *Phytomedicine* **16**, 181 (2009).
[16] J.M. Ford and W.N. Hait, *Pharmacol. Rev.* **42**, 155 (1990).
[17] D.L. Wu, F. Huang, and H.Z. Lu, *Ai Zheng* **22**, 441 (2003).
[18] S. Nobili, I. Landini, B. Giglioni, and E. Mini, *Curr. Drug Targets* **7**, 861 (2006).
[19] D.R. Hipfner, R.G. Deeley, and S.P. Cole, *Biochem. Biophys. Acta* **1461**, 359 (1999).
[20] C. Barthomeuf, J. Grassi, M. Demeule, C. Fournier, D. Boivin, and R. Beliveau, *Cancer Chemother. Pharmacol.* **56**, 173 (2005).
[21] F. Munoz-Martinez, C. Mendoza, I.L. Bazzocchi, S. Castanys, I.A. Jimenez, and F. Gamarro, *J. Med. Chem.* **48**, 4266 (2005).
[22] E. Lee and T.J. Marby, *J. Nat. Prod.* **48**, 326 (1985).
[23] M.H. Abd El-Razek, S. Ohta, and T. Hirata, *Heterocycles* **60**, 689 (2003).
[24] T. Mosmann, *J. Immunol. Methods* **65**, 55 (1983).