

Cytotoxicity of Selected Novel Chalcone Derivatives on Human Breast, Lung and Hepatic Carcinoma Cell Lines

Maryam Nakhjavani^a, Afshin Zarghi^b and Farshad H. Shirazi^{a,c*}

^aDepartment of Pharmaco-Toxicology, Shahid Beheshti University of Medical Sciences, Tehran, Iran. P.O.Box 14155-6153. ^bDepartment of Medicinal Chemistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran. P.O.Box 14155-6153. ^cPharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. P.O.Box 14155-3817.

Abstract

Cancer is considered as a challenging deadly disease and discovering or synthesis of new cytotoxic agents is a worldwide attempt. In this study, a group of recently synthesized chalcones, with the structure of 1,3-diarylprop-2-en-1-one having different COX-1 and/or COX-2 selectivities have been examined on human hepatocarcinoma (HepG2), lung carcinoma (A549), and breast adenocarcinoma (MCF-7) cell line, using Sulforhodamine B (SRB) assay. Briefly, cells were treated with 1-100 μ M of each compound for 72, 96 and 168 hours. In each case, a control row was set with the exposure of cells to compounds-free solvents. Median lethal concentration (LC_{50}) values (compared to controls) were calculated using regression fitness analysis on GraphPad Prism[®] software. Our results show that the subgroup possessing *p*-azido COX-2 pharmacophore seems to be more cytotoxic, while the cells seem to show more acquired resistance to them and the subgroup possessing a *p*-MeSO₂NH COX-2 pharmacophore is less cytotoxic, while the cells also acquire less resistance to them. In conclusion, considering the diversity in COX-1 or COX-2 inhibition among these compounds in each group, and also revealing no correlation between COX inhibition selectivity and cell death, it seems that selective inhibition of each isoenzyme doesn't cause substantial effect on toxicity potency. Further studies to determine the main mechanism(s) for these compounds induced cell death are encouraged.

Introduction

Novel compounds are the hope in cancer chemotherapy. Chalcone compounds are among the leading compounds for cancer chemotherapy with proposed anti-COX2 activities (1). Cytotoxicity assay of newly prepared agents is a "must" which is the focus of this study. Chalcones, with chemical structures of an aromatic ketone and an enone having a central

core, have become a favorable candidate for cancer therapy of different origins. They have been shown to have anti-tumorigenic activities (2, 3). Different cellular and molecular mechanisms have been proposed for various chalcones (4-6). Several chalcone structures have been synthesized and tested on human carcinoma cell lines with promising anti-proliferative results at micromolar to nanomolar concentrations (7, 8). However, less is known about the effects of these compounds on carcinoma cell lines and cells reactions to these compounds by the time of exposure.

* Corresponding author:

E-mail: f.shirazi@sbm.ac.ir

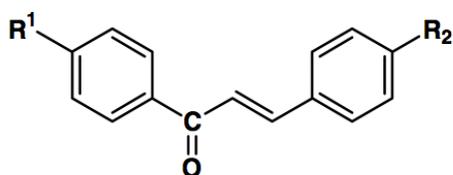


Table 1. Chemical structure of different 1,3-diphenylprop-2-en-1-one derivatives with various substitutions.

Compound	R1	R2
1	N ₃	H
2	N ₃	F
3	NHSO ₂ Me	H
4	NHSO ₂ Me	OMe
5	NHSO ₂ Me	F

Besides the role of cyclooxygenase-2 (COX-2) in inflammation, it has been shown that this isoenzyme plays roles in several cancers (9). Zarqhi *et al.*, in a study showed that chalcones with the chemical structure of 1,3-diphenylprop-2-en-1-one constitute a suitable scaffold for selective COX-2 inhibitory activity (10), which seems to be a promising target enzyme for cancer treatment (11, 12). In fact, a large amount of epidemiological and experimental evidences support a role for COX-2 in oncogenesis (13, 14). It is now recognized that COX-2 over expression promotes tumorigenesis, which can be suppressed by NSAIDs and COX-2 inhibitors, a phenomenon useful for the suppression of tumor progression (15).

In the present study, the cytotoxicity of a group of novel 1,3-diphenylprop-2-en-1-one chalcones are determined on three COX-2 expressing cell lines; human breast MCF-7 adenocarcinoma (16), human lung A549 adenocarcinoma (17), and human HepG2 hepatocarcinoma (18). Generally, COX-2 is highly expressed in aggressive metastatic breast cancers (*i.e.* growth and metastasis) (19) and in lung carcinoma (20). Some reports have also shown that NSAIDs inhibit the growth of hepatocellular carcinoma cell lines, a rationale to suggest their benefit in liver cancer therapy to be associated with liver carcinogenesis (21-23). To define the characteristics of the compounds-induced cytotoxicity and time based cellular

response to these effects, we conducted the present study testing the cytotoxicity of each compound at 72, 96 and 168 hours exposure time, using Sulforhodamine B (SRB) assay, which is an anionic dye that binds to intracellular proteins fixed in the plate and thus provides a sensitive index of cell viability (24).

Experimental

Materials

Compounds, otherwise specified, were purchased from Sigma®.

Chemicals

Different 1,3-diarylprop-2-en-1-one derivatives (Table 1) were synthesized in the department of medicinal chemistry of the school of pharmacy, Shaheed Beheshti university of medical sciences, as previously explained (25). Briefly, 1,3-diphenylprop-2-en-1-ones with an azido, or methansulfonamido substituent attached to the C-1 phenyl ring were prepared by one-step Claisen-Schmidt condensation.

Cell cultures

Human HepG2 hepatocarcinoma cell line (ATCC No. HB8065), human lung carcinoma A549 cell line (ATCC No. CLL-185), and human breast adenocarcinoma MCF-7 cells (ATCC No. HTB-22), were purchased from the Iranian Cell Bank of Pasture Institute, thawed and propagated for three passages in DMEM/F12 (Dulbecco's Modified Eagle Medium, Nutrient mixture F-12) (Gibco BRL®, USA) in flasks of 25 and 75 cm² surfaces (Orange®, Canada). Cells were plated at 6 × 10³ cells per well for HepG2 and A549 and 3 × 10³ cells per well for MCF-7 in 96-well flat-bottomed plates (Orange®, Canada) containing 180 μL of medium.

Cytotoxicity tests

The synthesized compounds were evaluated using the following protocol on above mentioned cell lines. The compounds were dissolved in dimethylsulfoxide (DMSO) as stock to make the final concentrations of 0-100 μg/mL by serial dilutions. Cisplatin as the positive control was dissolved in normal saline and further concentrations of 0-20 μg/mL were prepared by

Table 2. Physico-chemical properties of compounds 1-5.

Compound	Color and Crystal Form	Melting Point (°C)	Yield (%)	Formula
1	Cream crystalline powder	115-116	72	C ₁₅ H ₁₁ NO ₃
2	Cream crystalline powder	138-139	70	C ₁₅ H ₁₀ FNO ₃
3	Yellow crystalline powder	169-170	40	C ₁₆ H ₁₅ NO ₃ S
4	Yellow crystalline powder	132-134	44	C ₁₇ H ₁₇ NO ₄ S
5	Yellow crystalline powder	192-194	50	C ₁₆ H ₁₄ FNO ₃ S

serial dilutions. Following overnight incubation of cells, 20 μ L of each concentration was added to each well (final concentration of DMSO < 1%), control cultures received equivalent volumes of DMSO in the medium. All cultures were incubated at 37 °C in a 95% air/5%CO₂ humidified incubator. After 72, 96, or 168 hours of exposure, cell viability was determined by the protein binding SRB assay, according to the below protocol, for each well. The supernatant of each well was removed and adherent cells were fixed with 20% (w/v) cold trichloroacetic acid at 4 °C. After 30 minutes, the cells were washed with deionized distilled water and were air-dried. 50 μ L of Sulforhodamine B (0.4% (w/v) in 1% acetic acid) was exposed to cells for 30 minutes, and cells were washed with 1% acetic acid and air dried. Finally, 200 μ L of 10 mM unbuffered Tris (pH=10) was added to each well and the plates were shaken for 30 minutes. The optical density was measured at 540 nm.

Statistics

The median lethal concentration (LC₅₀) of each compound was calculated by plotting the percent of cell survivals vs concentrations of the test compounds using nonlinear regression

analysis in GraphPad PRISM® Software. All comparisons were performed in SPSS® software using General Linear Model, repeated measurement test. Comparison p values are less than 0.05 for all comparisons mentioned in the result sections and thus are not mentioned within the texts for the sake of simplicity.

Results and Discussion

Two different groups of compounds have been evaluated for cytotoxicity on cancer cells in this study which physico-chemical characteristics are presented in Table 2. The COX inhibition properties and cytotoxicities are presented below.

The subgroup of 1,3-diphenylprop-2-en-1-ones possessing a C-1 p-azido COX-2 pharmacophore

As previously described, these compounds exhibited good COX-2 inhibitory potency and selectivity, as shown in Table 3. Compound 1 shows moderate COX-2 inhibition with no inhibition of COX-1 at 100 μ M. On the other hand compound 2, which could inhibit both COX-1 and COX-2, has been shown to be 2.5 fold more potent inhibitor of COX-1 (25).

Table 3. *In-vitro* COX-1 and COX-2 enzyme inhibition assay data for 1,3-diphenylprop-2-en-1-one derivatives 1-5.

Compound	IC ₅₀ ^a		Selectivity Index (SI) ^b
	COX-1	COX-2	
1	>100	3.4	>29
2	4.2	10.0	0.4
3	3.0	3.2	0.9
4	1.0	10.0	0.1
5	3.3	>100	-

^a Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

^b *In-vitro* COX-2 selectivity index (COX-1 IC₅₀/COX-2 IC₅₀).

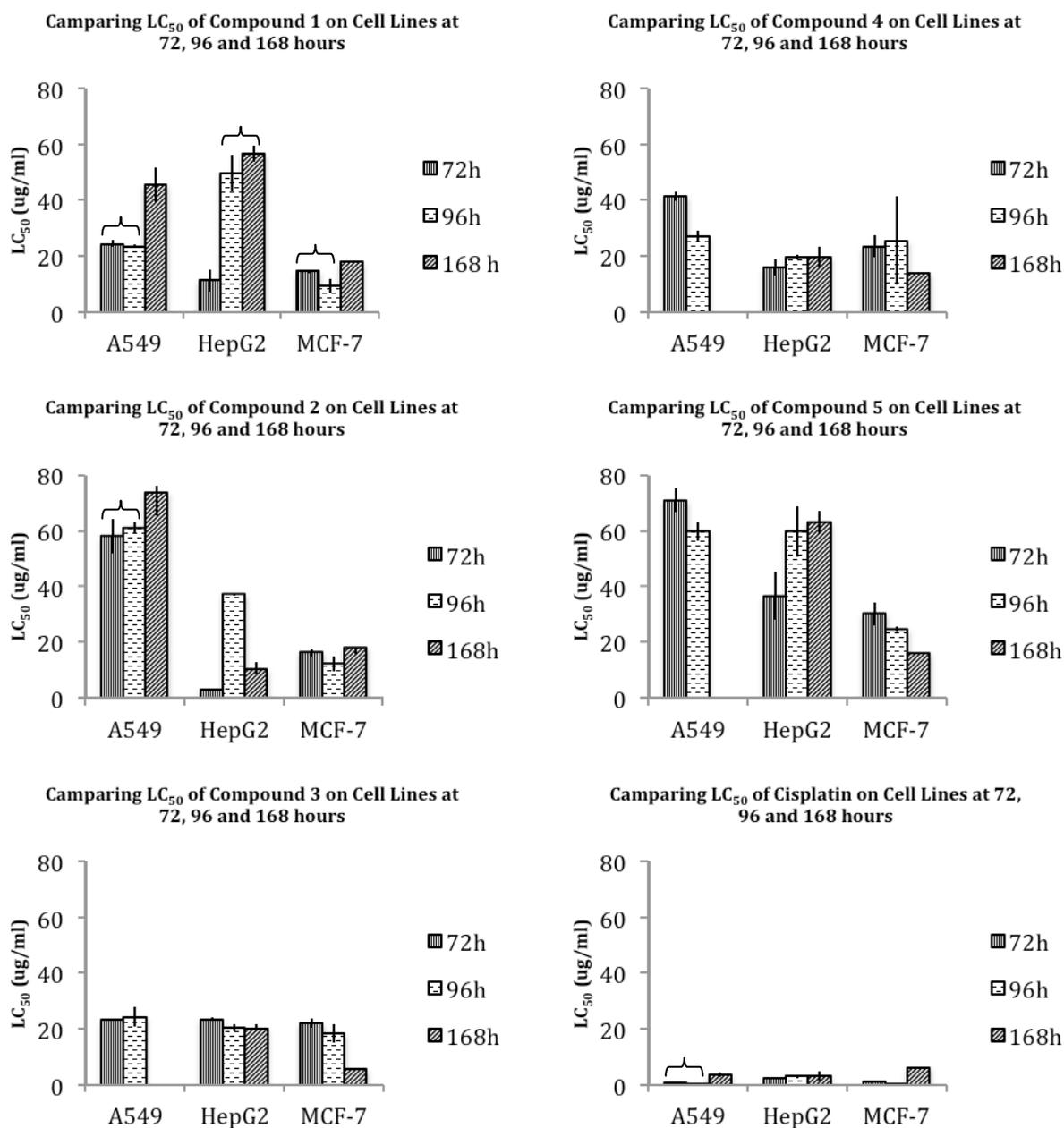


Figure 1. Comparing median lethal concentration (LC₅₀) of compounds 1-5 and cisplatin on human lung A549, hepatic HepG2 and breast MCF-7 carcinoma cell lines, at 72, 96 and 168 hours. p-value < 0.05.

Various studied cell lines seem to reveal similar patterns of cytotoxicity for these two compounds. An exposure time-dependent decrease in cytotoxicity is obvious for lung A549, hepatic HepG2, and breast MCF-7 cells with the exception of unusual cytotoxic pattern of HepG2 exposed to compound 2. Generally, from the cytotoxicological point of view, lung A549 cells seem to be less sensitive to these

compounds, while breast MCF-7 cells seem to be the most sensitive cell line (Figure 1). This sensitivity is well noticeable especially at 96 hours of exposure to compounds 1 and 2 (Table 4), which are comparable to the well-known chemotherapeutic agent cisplatin that is widely applicable in many cancers (26). However, it is important to notice that no statistically significant difference is obvious between the

Table 4. Median lethal concentration (LC₅₀) amounts ± standard deviation (SD) of compounds 1-5 and cisplatin on A549, HepG2 and MCF-7 cell lines, at 72, 96 and 168 hours.

Cell line	A549			HepG2			MCF-7		
	72 h	96 h	168 h	72 h	96 h	168 h	72 h	96 h	168 h
1	24.31 ± 1.30	23.42 ± 0.64	45.5 ± 6.17	11.19 ± 4.07	49.7 ± 6.42	56.52 ± 2.86	14.56 ± 0.69	9.482 ± 2.48	17.92 ± 0.19
2	58.09 ± 6.21	61.07 ± 1.85	73.95 ± 8.34	2.95 ± 0.19	37.09 ± 0.38	10.34 ± 2.03	16.12 ± 1.39	12.31 ± 2.65	18.14 ± 2.55
3	23.47 ± 0.31	24.06 ± 3.57	ND ^a	23.43 ± 0.61	20.55 ± 1.26	20.21 ± 1.42	22.12 ± 1.59	18.4 ± 3.30	5.60 ± 1.29
4	41.42 ± 1.80	27.09 ± 1.95	ND	15.85 ± 2.84	19.63 ± 0.80	19.73 ± 0.81	23.30 ± 3.97	25.52 ± 15.9	13.73 ± 3.73
5	70.96 ± 4.16	59.74 ± 3.22	ND	36.61 ± 8.53	59.75 ± 8.96	62.96 ± 6.85	30.19 ± 4.14	21.14 ± 3.07	15.98 ± 4.03
Cisplatin	0.81 ± 0.31	0.008 ± 0.005	3.38 ± 0.86	2.20 ± 0.20	3.03 ± 0.57	3.21 ± 1.56	0.96 ± 0.27	0.04 ± 0.12	6.14 ± 0.04

a: Not Determined.

cytotoxicity induced by compound 1 or 2, suggesting that COX selectivity might not be the dominant mechanism of cell death induced by these compounds.

3-2-The subgroup of 1,3-diphenylprop-2-en-1-ones possessing a C-1 p-MeSO₂NH COX-2 pharmacophore

In this group, compound 3 with an unsubstituted C-3 phenyl ring showed to have equipotent nonselective inhibition of COX-1 and COX-2, while substituting a *p*-OMe-phenyl resulted in a higher COX-2 inhibition (compound 4). On the other hand, C-3 *p*-fluoro substitution decreased the COX-2 inhibition and increased COX-1 inhibition as well as the total potency of compounds (especially in compound 5) (Table 3) (25). The general pattern of cytotoxicity of these compounds seems to be similar in most studied cases. Time does not seem to influence the cytotoxicity pattern of HepG2 in any cases, while an increase in cytotoxicity of long-term exposure is observable for A549 and MCF-7 (compounds 4 and 5) (Figure 1). In this group of compounds also, MCF-7 seems to be the most sensitive cell line. However, presenting compound 3 as the most cytotoxic agent claims again that selective COX-1 or COX-2 inhibition may not be considered as the main mechanism of cell death induced by these compounds.

Conclusion

In conclusion, the subgroup of 1,3-diphenylprop-2-en-1-ones possessing a C-1 *p*-azido COX-2 pharmacophore showed higher cytotoxicity, although the understudied cells

have later shown more acquired resistance to these compounds. On the other hand, although the subgroup with a C-1 *p*-MeSO₂NH COX-2 pharmacophore is less cytotoxic, however, cells show less resistance to these compounds. It is valuable to notice that all of these compounds share different amounts of COX-1 and COX-2 selectivity. Considering the diversity in COX-1 or COX-2 inhibition among these compounds in each group, and also revealing no correlation between COX inhibition selectivity and cell death, it seems that selective inhibition of each isoenzyme does not cause substantial effect on their toxicity potency. Despite COX-1 and/or COX-2 inhibition induced by these compounds, structure dependent cytotoxicity changes of these compounds, is independent to COX-1 and/or COX-2 inhibition. Therefore, further studies to investigate and determine the main mechanism(s) of cell death induced by these compounds and their related mechanisms for each compound is valuable and encouraged.

References

- (1) Shibata S. Anti-Tumorigenic Chalcones. *Stem. Cells* (1994) 12: 44-52.
- (2) Szliszka E, Czuba ZP, Mazur B, Sedek L, Paradysz A and Krol W. Chalcones Enhance Trail-Induced Apoptosis in Prostate Cancer Cells. *Int. J. Mol. Sci.* (2009) 11: 1-13.
- (3) Deb Majumdar I, Devanabanda A, Fox B, Schwartzman J, Cong H, Porco Jr JA and Weber HC. Synthetic cyclohexenyl chalcone natural products possess cytotoxic activities against prostate cancer cells and inhibit cysteine cathepsins *in-vitro*. *Biochem. Bioph. Res. Co.* (2011) 416: 397-402.
- (4) Pilatova M, Varinska L, Perjesi P, Sarissky M, Mirossay L, Solar P, Ostro A and Mojzic J. *In-vitro*

- antiproliferative and antiangiogenic effects of synthetic chalcone analogues. *Toxicol. In-vitro* (2010) 24: 1347-1355.
- (5) Hsu Y, Kuo P, Tzeng W and Lin C. Chalcone inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis. *Food Chem. Toxicol.* (2006) 44: 704-713.
 - (6) Kim T-H, Seo WD, Ryu HW, Seo HR, Jin YB, Lee M, Ji Y-H, Park KH and Lee Y-S. Anti-tumor effects by a synthetic chalcone compound is mediated by c-myc-mediated reactive oxygen species production. *Chem-Biol. Interac.* (2010) 188: 111-118.
 - (7) Modzelewska A, Pettit C, Achanta G, Davidson NE, Huang P and Khan SR. Anticancer activities of novel chalcone and bis-chalcone derivatives. *Bioorgan. Med. Chem.* (2006) 14: 3491-3495.
 - (8) Kong Y, Wang K, Edler MC, Hamel E, Mooberry SL, Paige MA and Brown ML. A boronic acid chalcone analog of combretastatin a-4 as a potent anti-proliferation agent. *Bioorgan. Med. Chem.* (2010) 18: 971-977.
 - (9) Razmi A, Zarghi A, Arfaee S, Naderi N and Faizi M. Evaluation of anti-nociceptive and anti-inflammatory activities of novel chalcone derivatives. *Iran. J. Pharm. Res.* (2013) 12: 153.
 - (10) Zarghi A, Arfaee S, Rao P and Knaus EE. Design, synthesis, and biological evaluation of 1, 3-diarylprop-2-en-1-ones: a novel class of cyclooxygenase-2 inhibitors. *Bioorgan. Med. Chem.* (2006) 14: 2600-2605.
 - (11) Bertino JR, Dannenberg AJ and Dubois RN. *Cox-2: A New Target for Cancer Prevention and Treatment.* Karger Medical and Scientific Publishers (2003) 37.
 - (12) Ghosh N, Chaki R, Mandal V and Mandal SC. Cox-2 as a target for cancer chemotherapy. *Pharmacol. Rep.* (2010) 62: 233-244.
 - (13) Doré M. Cyclooxygenase-2 expression in animal cancers. *Vet. Pathol.* (2011) 48: 254-265.
 - (14) Rizzo MT. Cyclooxygenase-2 in oncogenesis. *Clin. Chim. Acta.* (2011) 412: 671-687.
 - (15) Rüegg C, Zaric J and Stupp R. Non steroidal anti-inflammatory drugs and Cox-2 inhibitors as anti-cancer therapeutics: hypes, hopes and reality. *Ann. Med.* (2003) 35: 476-487.
 - (16) Larkins TL, Nowell M, Singh S and Sanford GL. Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression. *BMC Cancer* (2006) 6: 181.
 - (17) Qadri SSA, Wang JH, Redmond KC, O'donnell AF, Aherne T and Redmond HP. The role of Cox-2 inhibitors in lung cancer. *Ann. Thorac. Surg.* (2002) 74: 1648-1652.
 - (18) Cheng J, Imanishi H, Amuro Y and Hada T. Ns-398, a selective cyclooxygenase 2 inhibitor, inhibited cell growth and induced cell cycle arrest in human hepatocellular carcinoma cell lines. *Int. J. Cancer* (2002) 99: 755-761.
 - (19) Qiu D-K, Ma X, Peng Y-S and Chen X-Y. Significance of cyclooxygenase-2 expression in human primary hepatocellular carcinoma. *World J. Gastroenterol.* (2002) 8: 815-817.
 - (20) Bae SH, Jung ES, Park YM, Kim BS, Kim BK, Kim DG and Ryu WS. Expression of cyclooxygenase-2 (Cox-2) in hepatocellular carcinoma and growth inhibition of hepatoma cell lines by a Cox-2 inhibitor, Ns-398. *Clin. Cancer Res.* (2001) 7: 1410-1418.
 - (21) Prosperi JR, Mallery SR, Kigerl KA, Erfurt AA and Robertson FM. Invasive and angiogenic phenotype of mcf-7 human breast tumor cells expressing human cyclooxygenase-2. *Prostag. Oth. Lipid M.* (2004) 73: 249-264.
 - (22) Sun L, Zhang S, Jiang Z, Huang X, Wang T, Huang X, Li H and Zhang L. Triptolide inhibits cox-2 expression by regulating mrna stability in Tnf-A-Treated A549 cells. *Biochem. Bioph. Res. Co.* (2011) 416: 99-105.
 - (23) Li M, Chen F, Liu C-P, Li D-M, Li X, Wang C and Li J-C. Dexamethasone enhances trichosanthin-induced apoptosis in the Hepg2 hepatoma cell line. *Life Sci.* (2010) 86: 10-16.
 - (24) Al-Taweel AM, Fawzy GA and Perveen S. Cytotoxic flavonoid glycosides from rapistrum rugosum L. *Iran. J. Pharm. Res.* (2012) 11: 839.
 - (25) Zarghi A, Zebardast T, Hakimion F, Shirazi FH, Praveen Rao P and Knaus EE. Synthesis and biological evaluation of 1, 3-Diphenylprop-2-En-1-Ones possessing a methanesulfonamido or an azido pharmacophore as cyclooxygenase-1/-2 inhibitors. *Bioorgan. Med. Chem.* (2006) 14: 7044-7050.
 - (26) Hosseini SF, Wong P and Goel R. Interaction of cisplatin with cellular macromolecules: a fourier transform infrared spectroscopy Study. *Iran. J. Pharm. Res.* (2003) 2: 11-15.

This article is available online at <http://www.ijpr.ir>
